
# Program and Abstract Volume

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# CONFERENCE ON
# LIFE DETECTION IN EXTRATERRESTRIAL SAMPLES

**February 13–15, 2012 • San Diego, California**

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# Preface

This volume contains abstracts that have been accepted for presentation at the Conference on Life Detection in Extraterrestrial Samples, February 13–15, 2012, San Diego, California.

Administration and publications support for this meeting were provided by the staff of the Meeting and Publication Services Department at the Lunar and Planetary Institute.

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5:00 p.m. DIRECTED DISCUSSION

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**Chairs:** **Jeff Bada**
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11:15 a m. CONFERENCE DISCUSSION

12:00 p.m. *Meeting Adjourns*

**WE DID THIS BEFORE – THE LUNAR RECEIVING LABORATORY (1969 – 1972).**

Carlton Allen and Judith Allton
NASA Johnson Space Center, Houston, TX 77058 carlton.c.allen@nasa.gov and judith h.allton@nasa.gov

**Introduction:** The six Apollo missions to the lunar surface, between 1969 and 1972, returned 2,196 individual rock, soil and core samples, with a total mass of 381.69 kg. The astronauts selected samples, photographed the rocks and soils prior to collection, packaged them in uniquely identified containers, and transported them to the Lunar Module (Fig. 1).

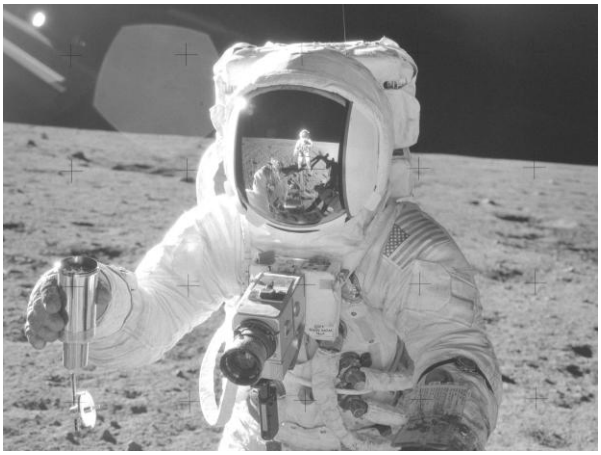

Figure 1. Apollo astronaut collecting soil sample (NASA photo AS 12-49-7278)

One of many unknowns prior to the Apollo landings concerned the possibility of life, its remains, or its organic precursors on the surface of the Moon. While the existence of lunar organisms was considered highly unlikely, a program of biological quarantine and testing was instituted starting with Apollo 11.

When the first missions returned to Earth the astronauts, their spacecraft, and the samples they collected entered biological isolation aboard the recovery ship. From there they were transported, still in isolation, to the Lunar Receiving Laboratory (LRL) at the Manned Spacecraft Center (now the Johnson Space Center).

The LRL had four specific functions [1]:

- Distribute samples to the scientific community
- Perform time-critical sample measurements
- Permanently store a portion of each sample
- Perform quarantine testing of the samples, spacecraft, and astronauts to assess the presence of indigenous life or biological hazards

The LRL comprised 8,000 $m^2$ of sample receiving laboratory, quarantine testing facilities, crew isolation area, gas analysis laboratory, and radiation counting laboratory. These last two laboratories were included because container head-space gas and cosmic ray-induced short-lived radiation measurements needed to be made rapidly, before samples could be released from quarantine.

Processing of lunar samples in a high-vacuum environment was initially deemed a science requirement because it preserved lunar-like conditions, as much as possible, and because no one knows how lunar materials would react with various gases. Processing in the vacuum glovebox was done for the Apollo 11 and 12 samples. However, manipulating and testing geologic samples in vacuum led to unacceptable levels of sample contamination. Subsequent sample processing and storage has been done in high-purity nitrogen gas, under positive pressure [2].

The lunar samples were processed through a sequence of steps which resulted in the following:

- Data upon which to base a release decision
- Preliminary scientific data upon which to base a sample distribution plan
- Portions of the lunar sample packaged for distribution to principal investigators
- Portions of the lunar sample sealed and protected for future experiments
- Time-critical experiments

On arrival at the LRL, sample boxes were moved through an airlock and through three decontamination chambers to sterilize the outside of the containers. They were then sent into a vacuum chamber where a technician punctured a diaphragm to draw off any gases. The sample was then passed on to a mass spectrometer to determine if the interior of the boxes had been contaminated by the Earth's atmosphere, and if any gases could be identified as being of lunar origin.

The boxes were opened in an environment free of terrestrial organisms. The nominal mode of operation called for opening the sample boxes in the special chamber described earlier which operated at a vacuum of $1.33 \times 10^{-4}$ $N/m^2$ ($10^{-6}$ mm Hg). An alternate mode employed the same chamber but with an atmosphere of

sterile nitrogen at a pressure slightly below atmospheric. A contingency mode was to open the containers in a Class III biological cabinet. Each lunar rock and portion of fine material was examined, photographed from six different angles, and observed visually through glass ports and through microscopes. A representative sample was committed to quarantine testing (Fig. 2).

Figure 2. Quarantine testing in the LRL (NASA photo S69-25713)

The preponderance of scientific work on the lunar sample was done by some 150 to 200 Principal Investigators throughout the world. Each investigator received a type and amount of lunar material suitable for their work and returned the residues to the LRL for further use by other researchers. A few of the Principal Investigators performed their experiments in the LRL during quarantine because of the time-critical nature of the data being sought [3].

Release of lunar samples was contingent upon meeting either one of the following:

- Biological safety tests upon representative portions of the samples.
- Sterilization of the sample by the use of dry heat during the quarantine period.

The biological safety tests were based on the "Comprehensive Biological Protocol for the Lunar Receiving Laboratory, developed by the Baylor University College of Medicine [4]. This "Baylor Protocol" involved tests of lunar material including bacteriology, mycology, virology-mycoplasma, mammalian animals, botanical systems, and invertebrate/lower vertebrate systems.

All protocols were designed to be completed within 30 days from the introduction of the sample to the laboratories. This was to be increased to 60 days in the event significant numbers of microbial contaminants were found in the sample. By 60 days, sufficient data would have been available to evaluate the requirement for second order testing. Quarantine testing revealed no evidence of life or hazard, and was discontinued after the Apollo 14 mission [5].

The amount of lunar sample used for quarantine testing during Apollo 11, 12, and 14 totaled 1.990 kg out of a total of 98.189 kg, or approximately 2%. An additional 0.269 kg was allocated from Apollo 15, 16, and 17 for post-quarantine biomedical follow-up testing.

Cost of construction, equipping and operating the LRL in 1969 and 1970 was about $24 million, with the most expensive features being the vacuum system and the low level radiation counting facility. At the height of mission operations 200 technicians worked in three shifts per day, supporting 100 NASA civil servants and visiting scientists. While quarantine testing was discontinued following Apollo 14, biological testing of samples continued through all six successful missions. The other functions of the LRL were continued through Apollo 17 – the final mission – in 1972, after which the laboratory was closed and eventually disassembled.

**References:** [1] McLane Jr., J.C., King Jr., E.A., Flory, D.A., Richardson, K.A., Dawson, J.P., Kemmerer, W.W., Wooley, B.C., 1967. Lunar receiving laboratory. *Science* 155, 525–529. [2] Allton, J.H., Bagby Jr., J.R., Stabekis, P.D., 1998. Lessons learned during Apollo lunar sample quarantine and sample curation. *Advances in Space Research* 22, 373–382. [3] Mangus, S., Larsen, W., 2004. Lunar Receiving Laboratory Project History. NASA/CR-2004-208938. NASA, Washington, DC. [4] Comprehensive Biological Protocol for the Lunar Sample Receiving Laboratory, 1967. [5] Johnston, R.S., Dietlein, L.F., and Berry, C.A., 1975, Biomedical Results of Apollo, SP-368, NASA Johnson Space Center, Houston, TX.

**CLEANING GENESIS MISSION PAYLOAD FOR FLIGHT WITH ULTRA-PURE WATER AND ASSEMBLY IN ISO CLASS 4 ENVIRONMENT.** J. H. Allton, NASA/Johnson Space Center, Mail Code KT, 2101 NASA Parkway, Houston, TX 77058, USA, judith h.allton@nasa.gov.

**Introduction:** Genesis mission to capture and return to Earth solar wind samples had very stringent contamination control requirements in order to distinguish the solar atoms from terrestrial ones. Genesis mission goals were to measure solar composition for most of the periodic table, so great care was taken to avoid particulate contamination. Since the number 1 and 2 science goals were to determine the oxygen and nitrogen isotopic composition, organic contamination was minimized by tightly controlling offgassing. The total amount of solar material captured in two years is about 400 micrograms spread across one $m^2$. The contamination limit requirement for each of C, N, and O was $<10^{15}$ atoms/$cm^2$ [1]. For carbon, this is equivalent to 10 ng/$cm^2$. Extreme vigilance was used in preparing Genesis collectors and cleaning hardware for flight. Surface contamination on polished silicon wafers, measured in Genesis laboratory is approximately 10 ng/$cm^2$ [2].

**"Start Clean – Stay Clean" a whole mission approach:** Contamination control was integrated into the Genesis mission from the very beginning: in mission design, in spacecraft design, in spacecraft cleaning and assembly, and in sample curation. This abstract focuses on cleaning and assembly of the payload, a science canister containing 300 solar wind collectors. The contamination sensitive solar collectors were isolated inside of a science canister in which organic materials (including lubricants and seals) were severely restricted and which was equipped with a molecular sorbent filter for pressure equalization upon re-entry. The mechanisms of the few moving parts were isolated and vented outside of the canister. The canister outer structure was fabricated from aluminum 7075 and interior structure from 6061. All interior aluminum surfaces were not anodized, to avoid the higher surface area and contamination introduced by the anodization process.

**Cleaning and assembly environment:** Canister components were cleaned and assembled inside of an ISO Class 4 laminar flow cleanroom [3]. Assembly was done by individuals totally enclosed in HEPA-filtered Teflon suits (Fig. 1). Gloved hands never touched cleaned hardware, to preclude transfer of any nitrile glove residue. Even smallest screws were installed using stainless steel tweezers.

**Cleaning process rationale:** The hardware was cleaned using ultrapure water (UPW) to avoid leaving any organic residue. [Note for future cleaning: UPW-may be especially useful if certain cleaning solvents are banned because of ozone depleting or greenhouse gas properties.]

Fig. 1. Engineers, enclosed in HEPA-filtered suits, assemble Genesis collectors into the science canister inside of an ISO Class 4 cleanroom at Johnson Space Center. Small pieces of cleaned aluminum foil are used to grasp the collector array, so that gloves never directly touch the hardware.

**Ultrapure Water (UPW):** UPW water (ASTM D5127-07), with metal cation and anion concentrations in the low parts per trillion, was chosen as the cleaning fluid for Genesis hardware because it was thought to leave very low residue. This ultrapure water, prepared by continuous filtration (at 0.04 µm particle size), irradiation with UV, and ion exchange, is characterized by a resistivity of 18 megohm-cm and a total oxidizable carbon (TOC) concentration of <10 ppb (typically <2 ppb). The Genesis ultrapure water system supplies 10 gal/min. The UPW is monitored continually for TOC content and particles down to 0.05µm in size (can detect single bacterial cells). Water this pure is a reactive solvent. It reacts with containers; thus, it is produced and used dynamically. Cleaning is done with flowing UPW, either in an ultrasonic cascade tank (Fig. 2) or megasonic wand for large items (Fig. 3). The resulting particle cleanliness level for two large items, the canister cover and base, is shown in Fig.4.

Fig. 2. Genesis array frame is cleaned with flowing UPW in a 30-inch diameter cascade tank enhanced with ultrasonic energy.

Fig. 3. Megasonically energized flowing UPW is used to clean large Genesis canister base.

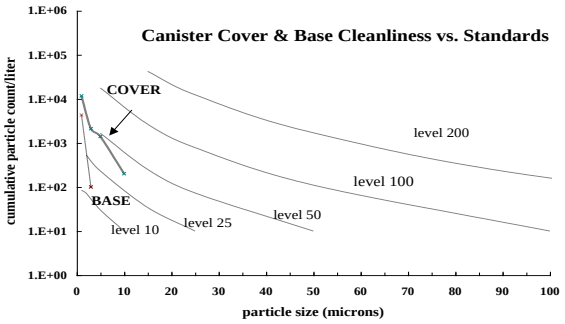


Fig. 4. Cleaning curves for large item canister cover and base are better than level 50 (IEST-STD-1246D). These items were cleaned as pictured in Fig. 3 and assessed by rinse water counts.

**Hardware Cleanability:** Clean design (coved corners, no blind holes, no inaccessible areas), smooth surfaces, and cleanable materials are required for rigorous cleaning. Stainless steel is very cleanable in UPW. Bare aluminum, as required to keep inorganic contaminants low, is reactive and challenging to clean with UPW. Fig. 5 shows effects of extended cleaning at high temperatures; therefore, aluminum parts were cleaned at 40° C.

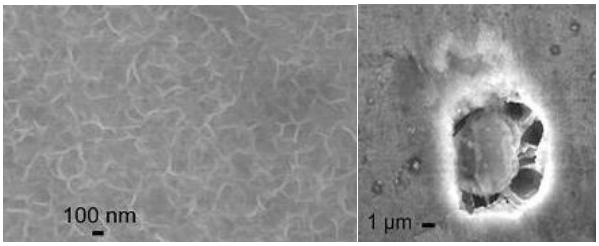


Fig. 5. Left is wrinkled texture of hydroxide formation on 6061 after 30 min. at 75° C (scale bar is 100 nm). Right is etched pit in 6061 after 30 min. at 50° C (scale bar is 1 µm).

**Keeping organic contamination low during assembly:** The organic lubricants and staking compounds were used inside of the canister only when demonstrated necessary and in extremely small amouts. For example, staking compounds were applied to exterior fasteners with the tip of a dental pick. A very small amount of Braycote lubricant was applied to the 94-inch circumference canister seal with a number 00 artist brush. Everytime a gloved hand came into contact with the Braycote container, the person left the lab and changed gloves.

**How well did we do?** Since Genesis solar wind samples were returned to Earth for analysis, an assessment of cleaning efforts can be made, at least for the organics. The hard landing in the Utah dry lakebed and breach of the canister containment cancels lab cleaning efforts. Even with the stringent controls on organics in the laboratory molecular contamination was acquired in orbit, most probably from offgassing of RTV and silicones. Ellipsometry measurements indicated a discontinuous film thickness of about 50 Å [4]. XPS measurements indicated silicone and fluorine. This film was thin and did not interfere with solar wind capture. The in-space UV exposure polymerized the film, requiring removal with UV ozone treatment [5].

**Success!** Genesis investigators, through persistence and ingenuity, have achieved the number 1 and 2 science goals of determining the oxygen and nitrogen isotopic composition of the Sun to high precision [6] [7]. The surprising (to many) oxygen abundances have directly challenged the basic solar system model and spurred new experimentation. Results have also been published for noble gases and some transition elements..

**Summary:**

- Clean assembly can be achieved inside of an ISO Class 4 laboratory.
- UPW is an effective solvent for cleaning particulates without leaving an organic residue.
- Even trace amounts of necessary lubricants and elastomers can migrate to sensitive surfaces at the monolayer level.

References: [1] Burnett D. S. *et al*. (2002) *Space Sci. Rev*. 105:509-534. [2] Allton J. H. and Burkett P. J. (2012) Abstract #6028 this volume.. [3] ISO 14644-1, Inst. Of Environ. Sci., Arlington Heights, IL. [4] Allton J. H. *et al*. (2006) *LPS XXXVII*, Abstract #1611. [5] Calaway *et al*. (2007) *LPS XXXVIII*, Abstract # 1627. [6] McKeegan K. D. *et al*. (2011) Science June 24, 2011, 1528-1532. [7] Marty *et al*. (2011) Science June 24, 2011, 1533-1536.

**TECHNICAL TENSION BETWEEN ACHIEVING PARTICULATE AND MOLECULAR ORGANIC ENVIRONMENTAL CLEANLINESS: DATA FROM ASTROMATERIAL CURATION LABORATORIES.**

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**Introduction:** NASA Johnson Space Center operates clean curation facilities for Apollo lunar, Antarctic meteorite, stratospheric cosmic dust, Stardust comet and Genesis solar wind samples. Each of these collections is curated separately due unique requirements. The purpose of this abstract is to highlight the technical tensions between providing particulate cleanliness and molecular cleanliness, illustrated using data from curation laboratories. Strict control of three components are required for curating samples cleanly: a clean environment; clean containers and tools that touch samples; and use of non-shedding materials of cleanable chemistry and smooth surface finish. This abstract focuses on environmental cleanliness and the technical tension between achieving particulate and molecular cleanliness. An environment in which a sample is manipulated or stored can be a room, an enclosed glovebox (or robotic isolation chamber) or an individual sample container.

**Room Environment:** Maintaining a particle-clean room is usually accomplished using HEPA (high efficiency particulate air) or ULPA (ultra low penetration air) filtered air maintained at higher pressure than the less-clean environment. Cleanrooms can be operated by diluting room air with filtered clean air (typically ISO class 6 or greater)[1]. The cleanest rooms are designed with laminar flow to control particle paths (typically ISO class 5 or less). Judicious use of cleanable materials and strict protocols can greatly improve cleanliness. JSC operates several astromaterial curation cleanrooms (Table 1).

**Airborne Molecular Contamination Levels in Genesis Laboratory:** Molecular contamination levels were measured in Genesis Lab air by exposing organic-free polished silicon wafers to lab air for 24 hours. The weight of organic species (with carbon chain length >7) sticking to a polished silicon wafer after exposure are presented in ng/cm$^2$ as yearly averages for 2001 through 2011 in Fig. 1. In general, the amount of airborne molecular contaminants decreased as activity levels decreased (year 2001-2004). Levels increased after repairs from flooding in 2010. Airborne species derived from RTV sealing compounds, used in the 54 ULPA fan filter units, and plasticizers are measured as a variety of cyclic siloxanes. Table 2 gives an example of other species detected. Observation of caprolactam is noteworthy. This nitrogen-containing species is an offgas product of nylon bags used to protect samples and supplies from particulate contamination.

**Glovebox and Storage Cabinet Environments:** Glovebox and nitrogen storage cabinets are purged continually with clean nitrogen. Thus, cleanliness depends upon an enclosure which is made of cleanable, low offgassing materials and of cleanable design and also upon pure purge gas. Enclosures using point-of-use (POU) filter/purifiers provide pure purge gas to samples. Genesis storage enclosure POU purifier/filters supply nitrogen with < 1 ppb $H_2O$, $O^2$, $CO^2$, CO and retain particles > 3 nm.

Gloveboxes are often used with inert purge gas to prevent unwanted chemical reactions to samples. However, any sample or hardware manipulation within a glovebox or robotic enclosure will generate particles abraded from the samples and tools inside the enclosure (particularly for rocky fines). Often a glovebox is assumed to be particle-clean. In fact, unless the particles are actively removed, this is not so.

If standard HEPA particle filtration technology is incorporated into the glovebox to remove particles, it is likely molecular contaminants are increased (mainly because filter sealants offgas cyclic siloxanes and other products).

Organic contamination was measured inside several curation stainless steel gloveboxes which are purged with pure nitrogen. These gloveboxes do not have active particle removal. Molecular contamination was measured as species adsorbed on a polished silicon wafer (witness plate) as described for Genesis laboratory air. An example of molecular species observed in one glovebox is shown in Table 3. Sources of organic contamination include offgassing from gloves and plastic sample bags. Offgas products from two glove materials are provided in Table 4.

Heat sealed bags are commonly used to protect samples and tools from particulate contamination; however, heat sealing produces molecular contaminants which can be absorbed on samples (Table 5). A combined heat sealing test of Teflon®, nylon, and polyethylene inside of nitrogen-filled glove box produced caprolactam (1300 ng/cm$^2$ from nylon), and lesser amounts of N,N-dibutylformamide, dibutyl phthalate, cyclo$(Me_2SiO)_8$, N,N-dibutylacetamide.

**Summary:** Techniques used to reduce particulate contamination or control atmospheric reactions may

increase molecular contamination. Some examples are given for planning purposes.

Table 1. JSC sample curation cleanrooms "at rest".

| ISO Class | Collection | Air Flow |
|---|---|---|
| 4 | Genesis solar wind | Laminar, vertical flow |
| 5 | Cosmic Dust, Stardust | Laminar, horizontal flow |
| 5 | Stardust | Dilution |
| 6 | Lunar | HEPA filtered supply |
| 6 | Space-exposed hardware | Dilution |
| 6-7 | Meteorite | HEPA filtered supply |

Figure 1. Genesis Laboratory molecular contamination levels 2000-2011. Vertical axis is ng organics/cm$^2$.

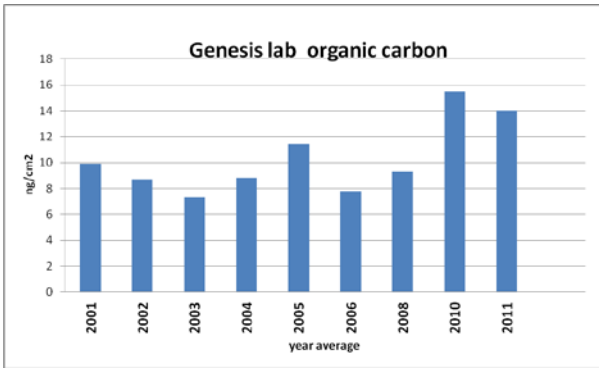


Table 2. Example of organics detected in Genesis lab air. These are semiquantitated results with detection limit 0.1 ng/cm$^2$ (TD-GC-MS, Balazs 2003).

| Species | ng/cm2 | Use |
|---|---|---|
| 2-(2-butoxy ethoxy) ethanol | 0.5 | Solvent used in paint, coatings |
| Di-isobutyl phthalate | 0.4 | Plasticizer |
| TXIB | 0.4 | Plasticizer |

Table 3. Example of molecular contaminants in a glovebox gas as measured by witness wafer. Semi-quantitated results with detection limit of <0.1 ng/cm$^2$ (TD-GC-MS, Balazs, 2000).

| Species | ng/cm2 | Use |
|---|---|---|
| TXIB | 0.2 | Plasticizer |
| N,N-dibutyl foramide | 1 | Unknown source. Species detected in enclosures containing tools & bags and gloved with neoprene |
| Diethylphthalate | 0.2 | Plasticizer |

Table 4. Offgas products from glovebox glove materials (GC-MS, JSC, 2000)

| Glove Material | Species |
|---|---|
| Neoprene | Carbonyl sulfide, propanone, acetaldehyde,butanal |
| Viton | Carbonyl sulfide, methanol,carbon disulfide, acetaldehyde |

Table 5. Gas extraction at temperature of melting, DSC, TX/GC/MS (JSC 1997)

| BagMaterial | Melting T, C | Species Offgassed |
|---|---|---|
| Teflon | 268 | none |
| Nylon | 221 | Caprolactam, 1-butoxy-2-propanol |
| Polyethylene | 110 | 1-pentene, pentane, heptane, 3-methylene-heptane |

**References:**

[1] ISO-14644-1, Class of Air Cleanliness, Institute of environmental Sciences and Technology

[2] Balazs Analytical Services, Fremont, CA.

**SEEKING EVIDENCE OF PAST LIFE ON MARS: THE POTENTIAL EVIDENCE THAT MAY BE CONTAINED IN RETURNED SAMPLES.** A.C. Allwood[1], [1]California Institute of Technology/Jet Propulsion Laboratory (4800 Oak Grove Drive, Pasadena, CA 93012).

**Introduction:** Returning samples for analysis on Earth is widely regarded as an essential step in the search for evidence of life on Mars. Accordingly, preparations are underway for a potential campaign to return samples from Mars, with primary science objectives centered on the search for evidence of past life [1]. However, the suite of material that would be returned from Mars would be limited to approximately 30 cores, weighing ~15-16g each—much less than the suite of materials typically involved in comparable life-detection studies on Earth [e.g. 2, 3]. The samples would also be selected under much more constrained field operational conditions. In light of these constraints, it is important to give considerable forethought to the types of evidence for past life that could potentially exist in such a sample suite and the types of in situ observations and approaches that could increase the chances that evidence of life would be captured in the samples.

**Approaches developed in studies of Earth's earliest biosphere.** A useful place to start this analysis is to look at studies of Earth's earliest biosphere. There are four different classes of biosignatures in the record of early life on Earth: microfossils, molecular fossils (biomarkers), chemical fossils (particularly isotopic abundances) and stromatolites (macroscopic sedimentary structures usually formed by microbes). To interpret a candidate biosignature, the general requirement is the same for all four classes: integrate multiple lines of evidence across multiple scales using combined field observations and sample analyses. This analysis includes documentation of the *inherent properties* of the particular candidate biogenic feature, as well as its *context*. Context consists of:

- The characteristics of the assemblage: a single biosignature is typically part of an assemblage
- The characteristics of the host rocks: environment of formation and subsequent history, based on a multitude of observations
- The spatial and temporal relationships between the candidate biosignatures, and between the biosignatures and the host rocks.

While the general approach described above is the same for all types of biosignatures, the specific approach is different for each class of biosignature. Because mission lifetime is highly constrained, understanding these differences is important for scientifically narrowing down the infinite possibilities for sample selection and performance of *in situ* measurements. The differences in approach come to light when certain key questions are posed:

- What are the *inherent* properties of the biosignature that would need to be observed?
- What *contextual* observations would be needed?
- Where is the necessary evidence likely to be detected: *in situ* or in returned samples, or both?
- Are the biosignatures likely to be contained within a single sample, or across multiple samples?

Two classes of biosignature are discussed and compared with regard to the questions posed above.

**Microfossils**: Cellular fossils are typically identified in thin section or through chemical separation of organic structures from the rock matrix of returned samples, and would almost certainly escape detection *in situ* with the instruments that are likely to be available on a rover. However, exceptions are possible, such as relatively large microfossils encased in large translucent crystals. These structures could potentially be seen, if not unambiguously interpreted, with a close-up imager on a rover. However, even if microfossils could be tentatively detected *in situ*, the inherent and assemblage characteristics of microfossils would need to be studied in returned samples.

The types of inherent properties that may help distinguish fossil cells from similar abiotic structures include such characteristics as cellular shape, signs of flexible but cohesive organic cell walls, nuclei, cell division, and isotopic abundances of organic material that make up the cell walls. At the assemblage scale, evidence of biogenicity may include community-like assemblages of cells, formation of microbial mats, deposits of draping biofilms, or remnants of extracellular polysaccharide (EPS) among the cells. Depending on the degree of preservation, typically at least several tens (if not hundreds) of candidate microfossils are studied in order to ascertain whether the structures are biogenic.

In addition to the inherent and assemblage properties of the microfossils, contextual information is needed. Some of that information would be derived from the microfossil-bearing samples themselves, but much of it would lie in the host rocks and would be acquired through both *in situ* observations and returned sample analyses. The measurements of context *in situ* would be done with no foreknowledge of the existence of microfossils, and would therefore need to focus on a general determination of the nature of the geologic deposit being sampled (e.g. chemical sediments, clastic sediments, or vein fills?) through multiscale observa-

tions of visible features, mineralogy and chemistry. These *in situ* interpretation of context would be verified through analyses of returned samples.

In summary, microfossils could exist in returned samples and multiple microfossils could exist within one sample. However, to confidently interpret them, morethan a single sample is needed. Multiple samples bearing microfossils would need to be collected to enable robust observations of inherent and assemblage properties. In addition, detailed *in situ* measurements supported by analyses of returned samples (not necessarily containing microfossils) would be needed to allow interpretation of the geologic setting.

**Stromatolites:** Stromatolites could be detected *in situ* with the instruments that are likely to be available on a rover. Both the inherent and assemblage characteristics of stromatolites could be studied extensively in the field through analysis of morphology and texture, and variability therein. Indeed, because stromatolites are large, many of their characteristics are only possible to study in the field and would not be captured in returned samples. However, critical detail is gained through laboratory analyses of returned samples, including thin section studies and geochemical analyses of minerals and organic deposits. On Earth, those samples are often large enough to contain a whole stromatolite, but smaller samples carefully selected from key locations within a stromatolitic deposit could encapsulate critical information on microtextures, geochemistry and organic deposits. Thus, samples would ideally be collected with these properties in mind, and *in situ* observations should focus on those properties that are too large to observe in a single sample, such as morphology, macroscopic textures, assemblage characteristics and the larger geologic context.

Stromatolites have few inherent properties that may help distinguish biogenic stromatolites from similar abiotic structures, for example, certain morphological and textural characteristics may be indicators of biogenicity. However, assemblage scale properties measurable *in situ* can be a rich source of information: morphological and textural variations correlated through space and time can provide evidence of biological influence on physical or chemical sedimentary processes occurring in the environment. Some of the most compelling properties of stromatolites, however, can be inherent properties that are not measured *in situ*, such as the distribution and character of organic deposits, isotopic abundances or microfossils.

Because the stromatolites are observable in situ, it is much easier to study them within their larger context. But, as with microfossils, the interpretation of context would rely on a combination of in situ observations and returned sample analyses. Insights to the origin of stromatolites lie in the correlation of stromatolite variations with variations in past environmental conditions.

**Summary:** Many different types of evidence for life may exist in returned samples. However, returned sample analysis alone is almost certainly insufficient for confident identification of past life and must be combined with *in situ* analyses to guide sample selection and provide geologic context. The process of selecting samples and providing context involves infinite possibilities, and consideration of the types of biosignatures that may be likely to occur in a given setting would help to significantly narrow down the range of possibilities.

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# Detection of Long Chain Bio-Polymers using Atomic Force Microscopy

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**Abstract:**

The Atomic Force Microscope (AFM) is demonstrated as a detector for long chain polymers and soft organics on mineral surfaces. Long chain (linear) polymers have been proposed as a general bio-markers [1-3]. The AFM provides very sensitive detection of polymeric strands using the force spectroscopy mode of operation [4]. In this mode, the force is measured as the AFM tip makes intermittent contact with a polymeric sample. As polymer strands are pulled from the surface onto the AFM tip, a characteristic detachment signal is measured in the force-distance plot.

AFM force spectroscopy provides polymer length, folding information and is capable detecting single molecules. AFM force spectroscopy of synthetic proteins, fossils surfaces and algae on mineral surfaces are presented as test examples.

AFM instruments have flown on two space missions, the Phoenix MECA experiment and the Rosetta MIDAS experiment [5,6]. In addition to providing high resolution imaging, this work demonstrates a powerful new application of the AFM that is relevant to astrobiology missions.

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**Planning for the Analytic Environment to Conduct Life Detection Experiments on Samples Returned from Mars: Observations and Issues** D. S. Bass[1], D. W. Beaty[1], C. C. Allen[2], A. C. Allwood[1], L. E. Borg[3], K. E. Buxbaum[1], J. A. Hurowitz[1] and M. D. Schulte[4] [1]California Institute of Technology/Jet Propulsion Laboratory (4800 Oak Grove Drive, Pasadena, CA 93012) Deborah.Bass@jpl.nasa.gov, [2]NASA Johnson Space Center (Houston, TX 77058) [3]Lawrence Livermore National Laboratory (Lawrence Livermore National Laboratory 7000 East Ave, Livermore, CA 94550), [4] NASA Headquarters (MS 3V71, 300E St SW, Washington, DC 20546)

**Introduction:** If samples were to be brought from Mars to Earth by a robotic sample return mission, the samples would initially reside in a specialized facility capable of highly reliable biocontainment. At a minimum samples would need to be sterilized or assessed for any potential biohazard [1]. In an alternate model, a significant capability for scientific research might be designed into the facility so that a variety of analyses could be accomplished while keeping the samples in biocontainment. What does this imply for researchers who might aspire to study samples returned from Mars? Further, what would it mean to the planetary protection community to consider alternate approaches to meeting the requirements for biohazard assessment as set forth in international policy?

*Definitions:* Biocontaiment laboratories are classified in biosafety levels (BSL) 1 to 4. BSL-1 laboratories are suitable for studies involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment [2]. At the other extreme, BSL-4 laboratories are designed to contain and allow the manipulation of dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections, and agents which cause severe to fatal disease in humans for which vaccines or other treatments are not available, such as, Marburg virus, Ebola virus, Lassa fever, smallpox, and various other hemorrhagic diseases. BSL-4 laboratories are designed within larger facilities with lower levels of containment. In addition to the designation of safety levels for laboratories as a whole, there is also a classification scheme for cabinets that are used to conduct experiments on different types of pathogens [3]. Biosafety Cabinets (BSC) of Class II and above use airflow and HEPA filtration to minimize aerosol contamination of the surrounding environment. Class III enclosures are gas-tight, and all materials enter and leave through a dunk tank or double-door autoclave. Gloves attached to the front prevent direct contact with hazardous materials. These custom-built cabinets often attached to each other, and the lab equipment installed inside is usually custom-built as well. In some BSL-4 laboratories, the primary barrier for containment is the BSC. The suit covering the personnel only improves the containment, and is considered a secondary barrier.

Formal advice to NASA and others who would undertake sample return from Mars has been that the highest containment standard—Biosafety Level 4—would be required for study of unsterilized Martian samples [1]. This applies equally to a life detection and biohazard assessment that would be a mandatory prerequisite for ultimate release of samples from biocontainment. In addition to the highest levels of containment, returned Martian samples would be subject to extremely stringent levels of contamination control to protect the scientific integrity of the samples. This is one of the unique aspects of a Mars Sample Receiving Facility (SRF)—the combination of high level biocontainment and combined with contamination control. NASA scientists and engineers have visited a number clean room and BSL-4 laboratories to understand the specific designs and choices of laboratories that might guide the design and development of a Mars SRF. All visited laboratories share some common strategies, but they incorporate a number of differences depending upon the particular facility's charter.

On September 27, 2011, a group of scientists representing NASA's Mars Exploration Program visited the National Biodefense And Countermeasures Center (NBACC) to understand some of the key design challenges. The Department of Homeland Security built the NBACC laboratories to enhance its capabilities to protect the American public from bioterrorism. This research facility is located within the National Interagency Biodefense Campus at Ft. Detrick, Maryland. More information regarding NBACC can be found at http://www.bnbi.org/.

**Observations of Existing Biosafety Labs**:

• *Significant experience in working with hazardous materials.* While the notion of working with pathogens and biosafety levels may be new for many at NASA, working in these environments (BSL-2, 3, 4) is relatively commonplace, with an entire industry devoted to the design, construction, and operation of these facilities. NASA should continue to consult with NBACC and others who operate BSL-4 laboratories, using them as a resource to avoid duplication of research for Mars Returned Sample Handling (MRSH) and sample handling protocols. For example, the safe transport of samples within and between BSL-4 laboratories – a recognized requirement for Mars

Sample Return – is well established in the biosafety community.

• *Modular designs.* Construction details of the NBACC labs of particular interest to the NASA science team included access chambers and a modular design. For example, NBACC's facility has substantial access chambers adjacent to the laboratory spaces for moving large equipment in and out of the facility to address the issues of equipment maintenance and biocontainment. Also, the NBACC design did not attempt to predict all future uses, but rather adopted a modular approach in which a given space could be modified to enable a particular task to be completed. This approach may be useful for NASA since the particular measurements (and hence instruments, preparation procedures, and curation restrictions) would likely not be understood until the samples are examined in some detail. These sorts of design choices would be made during the construction phase of a Mars SRF.

• *Laboratory Sterilization.* The NBACC containment labs are clean from the point of view of live biology, since a significant portion of their purpose is to search for trace organisms. The labs are periodically sterilized by introduction of vaporized hydrogen peroxide (VHP) or formaldehyde gas. Equipment is passed through an autoclave on its way in and out of some laboratories. Typically, BSL-4 labs can tolerate higher background of dead organisms and organic molecules than will be acceptable for MRSH. Furthermore, for Mars Returned Sample Handling, sterilization procedures may need to be re-examined. For example, there are organisms on Earth that can survive autoclaving. Also, the Earth-based standard techniques for whole room decontamination (*e.g.*, vaporized hydrogen peroxide or formaldehyde) could be a concern because it is unknown whether putative Martian organisms can withstand or perhaps even metabolize hydrogen peroxide. [4] While 'extreme' organisms are unlikely to be pathogenic, if sterilization is required, it would need to be definitive. This may be at odds with equipment requirements as well as organic compound detection, etc., which are already recognized issues.

• *Cleaning.* Another take-home message is the difficulty of cleaning a BSL-4 facility to the class 100 cleanroom conditions necessary to conduct inorganic analysis of the type commonly applied to planetary materials. One possibility that could be explored, is building a cleanroom space within the BSL-4 facility for this type of analytical procedure. The combination of stringent cleanroom and biosafety reqirements in a single facility is not common, and would require extensive development, testing and certification. Alternatively, inorganic analyses could be completed on samples outside the BSL-4 facility, if suitable sample sterilization protocols could be developed.

• *Instrumentation needs.* The visiting science team could not envision how it would be possible to clean these labs to the point that scientists could conduct experiments regarding trace evidence for the signs of unknown life with Martian samples on open benches. The required kind of environment would only be credibly created inside biosafety cabinets. Therefore, there is an as-yet undefined relationship between the instrumentation (that will do the life detection), the clean environment, and the containment barrier. A significant program of research, development and certification will be required to meet these key science requirements.

• *Stand-alone vs. Add-On.* After the required planetary protection testing has been executed, biocontainment may not be necessary. As a result, NASA would not wish to have built a significant facility that would be abandoned after several years of use. As a result, NASA should consider building a facility that might have capabilities beyond bio-containment (*e.g.* curation capabilities for future study).

**Conclusions:** While the NBACC laboratories and other BSL-4 labs satisfy some of the requirements of a sample receiving facility, the primary mission of BSL-4 labs and their goals are inherently different. Addintionally, NBACC also deals primarily with fairly well-known organisms. NASA needs to scrutinize carefully the advice and expertise from existing biosafety laboratories, and be cognizant that there will be issues during sample return evaluation and curation that will require independent thinking and solutions.

It is clearly necessary to begin the planning for an SRF early in the Mars Sample Return Campaign. Previous studies conducted by the National Research Council [5] recommend that planning for an SRF begin at least seven years prior to the return of the Martian samples.

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**Status Report of a Joint Science Working Group for a Proposed 2018 Joint Mars Mission.** D. W. Beaty[1], G. Kminek[2], the 2018 Joint Science Working Group (Allwood, Abby; Arvidson, Ray; Borg, Lars; Farmer, Jack; Goesmann, Fred; Grant, John; Hauber, Ernst; Murchie, Scott; Ori, Gian Gabriele; Ruff, Steve; Rull, Fernando; Sephton, Mark; Sherwood Lollar, Barbara; Smith, Caroline; Westall, Frances; Pacros, Anne; Wilson, Mike; Meyer, Michael; Vago, Jorge), the Joint Operations Scenario Working Group (Bass, Deborah; Joudrier, Luc; Allwood, Abby; Boyes, Ben; Francescetti, Paola; Hurowitz, Joel; Laubach, Sharon; Loizeau, Damien; Pacros, Anne;), and the Joint Instrument Team (Feldman, Sabrina; Trautner, Roland) [1]California Institute of Technology/Jet Propulsion Laboratory (4800 Oak Grove Drive, Pasadena, CA 93012), [2]ESA-ESTEC (Keplerlaan 1, 2200 AG Noordwijk, The Netherlands)

**Introduction:** NASA and ESA have started discussions about a joint Mars mission proposed for launch in 2018. This rover mission would pursue *in-situ* science and caching of samples for potential subsequent return to Earth.

A Joint Science Working Group (JSWG) and a Joint Engineering Working Group (JEWG) have been formed in June 2011 to support definition of the proposed 2018 mission concept. The JSWG is composed of scientists from the US, Canada, and Europe and supported by the Joint Operations Scenario Working Group (JOSWG).

The main tasks of the JSWG are to:

- Establish the scientific objectives for the proposed joint mission;
- Establish scientific requirements to meet the science objectives;
- Establish a reference surface mission scenario consistent with the science objectives and requirements.

The deliberations of the JSWG are based on the assumption that the ExoMars Pasteur Payload, including the 2-meter deep drill, would be part of the proposed joint mission and take into account the MEPAG ND-SAG and MEPAG E2E-iSAG reports.

A report of this working group will be delivered to the Joint Mars Exploration Executive Board (JMEB) for their consideration by the end of January 2012. This presentation will provide a summary of this report.

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**TELE-ANALYSIS OF RETURNED MARS SAMPLES: AN OPPORTUNITY FOR NASA?** D.F. Blake[1] and L.F. Allard[2], [1]Exobiology Branch, MS 239-4, NASA Ames Research Center, Moffett Field, CA 94035, david.blake@nasa.gov, [2]Materials Science & Technology Division, Oak Ridge National Laboratory, 1 Bethel Valley Rd., MS 6064, Oak Ridge, TN, allardlfjr@ornl.gov

**Introduction:** Returned samples from Mars will be the most valuable scientific materials ever obtained, eclipsing by far the lunar soil and rocks returned during the Apollo program. In addition to the inestimable value of returned samples from Mars, there will be other reasons why scientifically, politically and socially its curation, accessibility and analysis will be unique in the history of science:

- The sample is from another planet which could have harbored life, and there will be legitimate concerns about public health and safety. How will we satisfy public fears of back-contamination - an "Andromeda Strain" event?
- A principal research interest in the sample involves whether extant life, remnants of extinct life, or pre-biotic organic compounds are contained within it. Because the Earth is teeming with life and its byproducts, the samples will require special protection from the Earth environment.
- A Mars sample return mission in the current (and forseeable) fiscal climate will be of necessity an international collaboration. Shared cost carries with it the expectation of shared reward in the form of equal access to samples.
- The gold standard of empirical science is repeatability. An analytical result reported by a single research team (especially an observation pertaining to extraterrestrial life) must be validated by another research group in the same or an equivalent sample. How can a one-in-a-million discovery be repeated if you have fewer than a million samples?
- The instruments used for analyzing returned samples will have to be upgraded and modified to a degree we have not experienced before (contamination free, parsimonious with sample material).
- Sample handling and sample preparation will be unique for each type of sample and each technique. How will samples be prepared to minimize waste and redundancy?

**Telescopes, Microscopes and Mars Rovers:** "Tele-astronomy" "Tele-microscopy" and "Tele-robotics," are methods of "instrument" control that are already in use within some areas of NASA and DOE, and are being adopted by the private sector as well. For example, tele-robotic operation is a requirement when the instrument is a rover operating on Mars. Beginning with the Mars Exploration Rovers *Spirit* and *Opportunity*, remote operations have become increasingly more user-friendly, to the point where Payload Uplink and Downlink commands can be accomplished using a laptop computer virtually anywhere in the world (although the actual spacecraft commands are still uplinked by controllers at JPL).

Major earth-based research telescopes are fully staffed and automated to optimize instrument usage and availability via tele-astronomy methods. Astronomers who wish to have observing time write peer-reviewed proposals and when observing time is granted, operate the telescopes from control rooms far from the mountain tops where the instruments are located. Data are collected in real time and analyzed offline.

For characterization of returned Mars materials, the concept of tele-analysis via methods such as tele-microscopy is a logical extension of NASA's experience with tele-robotics and tele-astronomy. Tele-microscopy methods were pioneered by the Department of Energy through the Materials Microcharacterization Collaboratory project among national laboratory (Oak Ridge, Argonne and Lawrence Berkeley National Laboratories) and university (Univ. of Illinois) partners in the 1990's. Microanalytical methods require a wide array of instruments for specimen preparation and data collection, and represent the most operator-intense scientific processes that will be involved with analysis of Mars materials. Much of the hardware and software required for the remote operation of state-of-the-art Analytical Electron Microscopes (AEMs) is routinely adapted to modern microscopes by the manufacturers. Modern AEMs cost in the neighborhood of ~$2-5M, require >$100K in annual maintenance contracts, and need technicians and specialized sample preparation equipment for optimal use. The most advanced instruments require specialized buildings to provide the quiet environment needed to routinely achieve their specified operation levels.

**How Should Sample Return Science be Accomplished?** Returned samples must be handled in such a way that the public health is protected. Returned sample science should be available equally among the international science community, and samples should be analyzed in a way that false positives and false negatives are minimized or eliminated and so that important observations can be repeated.

*The Curatorial Facility.* The curatorial facility should be permanently staffed, and contain all of the

instruments and infrastructure necessary for sample curation, sample preparation and sample analysis. The facility should be built to P-4 or whatever biohazard level is deemed sufficient to prevent forward or back-contamination of the sample. Once returned samples are put in the curatorial facility, they will never (or rarely) be removed.

*The International User Community.* The scientific community with access to the returned samples would include anyone who has written a successful peer-reviewed proposal. The only required equipment at the PI's location should be a computer and a fast data link.

*Returned Samples and their Analysis.* Samples should be prepared in the curatorial facility by the permanent technical staff, and loaded into and removed from the analytical instruments by them. Data from the analyses are downlinked to the PI as the observations are made, as well as kept in an archive at the curatorial facility. Should the same observation be proposed by another researcher (either for another purpose or for validation of an earlier analysis), that researcher could reacquire the same location on the sample and perform the same analysis with the same equipment. Each set of observations obtained by a researcher should be covered by "Rules of the Road" document which allows priority to that researcher for those data for a set period of time (e.g., six months). Returned sample science could be performed by individual researchers with only a few $K of investment in a computer and data analysis software.

**What Are the Benefits for NASA?** NASA engineers and scientists will work together to create new robotic technologies and applications for science. This will have a variety of benefits: Scientists will become more familiar with their instruments and the measurements they make. This will have the effect of improving the technology, and creating more links between the engineering community and the scientific community, something that is relatively rare at the present time. The specialized sample handling and sample preparation techniques that will be necessary for returned samples will be tested and in place when the samples return. Scientific – technical collaborations will benefit spaceflight instrument development, and produce spinoff applications in the private sector. In the long run, there will be more accessibility of science and research to institutions that have been traditionally left behind as a result of the high cost of research facilities. Early adoption of tele-analytical science will forge collaborations between scientists of different countries and institutions and will establish trust among NASA, European Space Agency and other funding partners that science return will be shared equally.

The tele-microscopy will be shown as an example of what is already being done in DOE labs such as Oak Ridge National Laboratory. The Materials Microcharacterization Collaboratory project could be used as a starting point for designing and developing the infrastructure for Mars returned sample science.

**THE EFFECT OF CARBONATE ON THE DETERMINATION OF $\delta^{13}C$ BIOSIGNATURE IN LOW-ORGANIC LOW-CARBONATE SOILS FROM THE ATACAMA: RELEVANCE TO THE SEARCH FOR PAST LIFE ON MARS.**
R. Bonaccorsi[1,2], C. P. McKay[1], and A. P. Zent[1] - [1]Space Science Division, NASA Ames Research Center M.S. 245-3-1000 Moffett Field, CA 94035 USA. [2]SETI Institute - 189 Bernardo Avenue Mountain View, CA 94043 Rosalba.Bonaccorsi-1@nasa.gov.

**Introduction:**

A key goal of the next decade planetary missions is to determine whether life developed on any planetary body in the solar system other than Earth. The 2011 Mars Science Laboratory (MSL) and the ESA 2016/18 Pasteur ExoMars missions will seek information on the geological and biological history of Mars at landing sites with the expected highest preservation potential of organics [1].

Detailed information on the abundance and $^{13}C/^{12}C$ isotope ratios ($\delta^{13}C$) of inorganic (IC) and organic carbon (OC) mixed in geological materials is essential for modeling planetary formation and geochemical evolution and, in particular, for the search for past/present life on Mars.

On Mars carbonates have been detected at 1-5% as background component, or even in higher amounts in localized settings e.g., at Nili Fossae [5-8]; these carbonates could be mixed with the organic-poor Martian soil.

The MSL Sample Analysis at Mars (SAM) payload will characterize the $\delta^{13}C$ of the total $CO_2$ gas evolved from combusted/pyrolized geological samples [12] and bearing a mixed IC (less negative $\delta^{13}C$) and OC (more negative $\delta^{13}C$) signature. As a result, the instrumental capability of unraveling IC and OC from measured total carbon pools (TC=IC+OC) will be challenged by amount and type of carbonate minerals in geological samples.

In this context, of most relevance is the understanding of carbonate removal effects on the $\delta^{13}C$ signature of environmental samples characterized by a Low Organic Carbon/Low Carbonates content (herein referred as to LOCC).

**Novel aspects of " LOCC Mars-like" materials:**

We first present Elemental Analyzer-Ratio Mass Spectrometer (EA-IRMS) data from carbonate removal/combustion tests on a suite of LOCC soil analogs from the "Mars-like" [e.g., 1-2, 11] Atacama Desert, Chile (Figure 1). As these soils contain very low levels of refractory organics (at the 0.1% level), they have been suggested to represent key analogs of possible Martian organics [e.g., 1-2]. However, their amount of carbonate carbon have been overlooked.

In the second part of this paper, we compare LOCC soil from the Atacama with other mineralogically and biologically diversified LOCC environments worldwide. These includes: *a*) cyanobacteria-colonized/non colonized quartz sandstone (Antarctic Dry Valleys) and rhyolitic beach deposits from the peri-Antarctic Shetland Islands; *b*) clay-rich/Fe-oxyhydroxides-rich units from the hyperacidic drainage system of the Rio Tinto (Spain); and *c*) sub-lava-flow fossil soil rich in nontronite clay (Hawaian Islands) and subaerial analogues from the Mojave Desert.

**Background & Study Site:** The Atacama desert extends across 1000 km (30ºS to 20ºS) along the Pacific coast of South America (Figure 1) within the rain shadow of the Andes. This desert is one of the oldest and driest deserts on Earth, has been considered a renowed analog model for constraining the limits of life/ or its preserved remnants on Earth as well as on an early/ present-day Mars [1-2].

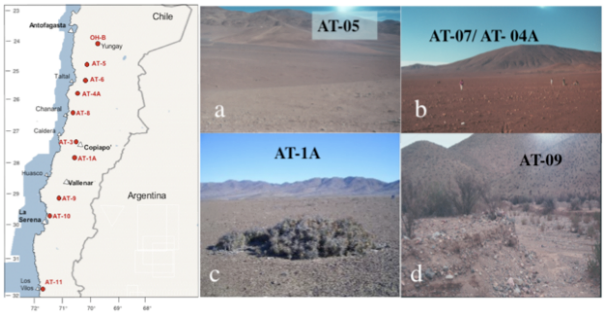


**Figure 1.** Left: Map showing location of tested samples. Right-hand side: (a) Representative hyperarid soil (~24ºS and ~0.5mm/y rain); and (b-d) arid sites (~32ºS and >120 mm/y rain).

**Approach and Methods:**

The elemental CN and naturally occurring stable isotopes of TC and OC were determined with a Carlo Erba NA-1500 Elemental Analyzer coupled with a Finnigan Mat Delta Plus XL IRMS instrument. Up to 50 to 100 ml of acid was added to the sample into silver boats in small increments until effervescence stopped and oven dried at T<60°C. Several cycles were repeated until no release of $CO_2$ was observed from the samples. To determine the efficiency of carbonate carbon removal sub samples were independently tested with sulfuric acid (2N $H_2SO_4$) and hydrochloric acid (1N HCl), which are commonly used for carbonate removal [9-10]. Solid samples were flash combusted (>1600°C) in a stream of pure $O_2$ (He car-

rier) to complete oxidation of organic matter and inorganic carbon [e.g., 9] to gases reduced and chromatographically separated ($CO_2$, $N_2$) prior injection into the MS unit.

**Results and Conclusions:**

*1. Total carbon, organic and inorganic carbon.* Figure 3a plots the compositional field of total and organic carbon pools with respect to the total amount of carbonate minerals (as %$CaCO_3$). Samples fall into three different compositional fields of the OC-carbonate diagram. 1. Extremely low OC (<0.06 wt.%) – low $CaCO_3$ (< 0.1-1 wt.%); 2. very low OC (< 0.1%) – low $CaCO_3$ (<1 wt.%); 3. relatively low OC (~0.1-1 wt.%) – $CaCO_3$ (~1-3 wt.%); and 4. higher OC (~1-2 wt.%) – $CaCO_3$ (~10-20 wt.%). The carbonate amount is directly correlated with the total carbon in samples i.e., open red circles symbols ($R^2$ = 0.97).

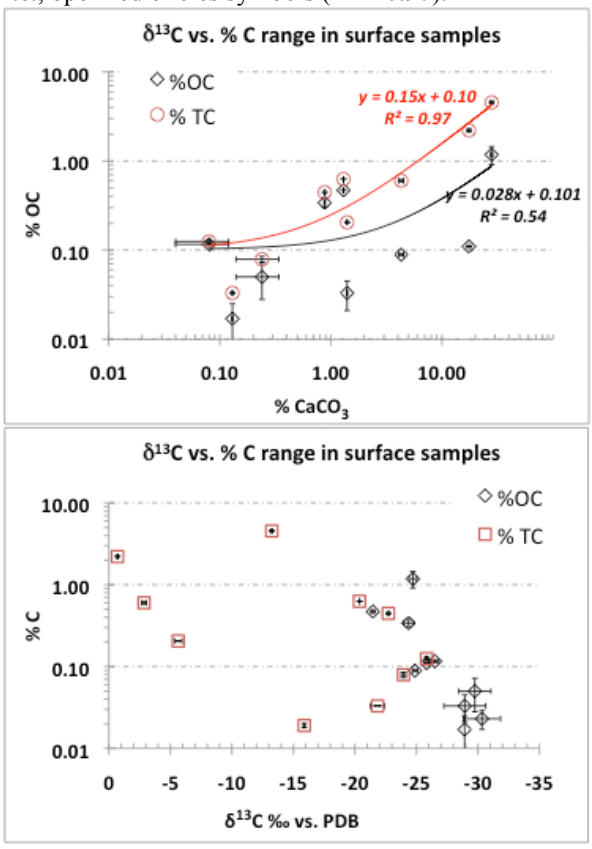


**Figure 2ab.** Organic geochemistry results in acid processed (%OC) and not processed bulk samples (%TC) from the Atacama Desert.

*2. Stable isotopic composition of total and organic carbon.* In Figure b we distinguish two different groups of soil based on their OC% vs. $\delta^{13}$C-OC composition. 1) Soil with an extremely low amount of OC (<0.06 wt.%) and more negative values of $\delta^{13}$C-OC (–30‰ to –28‰); these are from the plant-barren and low in active bacterial biomass e.g., $10^5$ - $10^6$ cell/gram [11]; 2) arid soils with a higher amount of OC (<0.6 wt.% to 2.2wt.%) and less negative OC- $\delta^{13}$C (-21.5‰ to -26.5‰); these surface soils are mostly vegetated and contain higher living microbial biomass e.g., $10^7$ - $10^8$ cell/gram [11]. The less negative values of TC ($\delta^{13}$C-TC ~15‰ to ~ -1 ‰) reflect a consistent fraction of carbonates relatively to the total carbon fraction (28% to 95%).

*3. Effect of carbonate removal on bulk organic and inorganic* $\delta^{13}$C *signature.* Results from carbonate removal experiments show that the carbon isotopic composition can significantly shift (from 2‰ up to 5-7‰) even for small amounts of carbonate (<0.01-0.1%) left in the soil matrix. Understanding and mitigating this effect is necessary for the correct interpretation of bulk organic geochemistry data in organic-poor/carbonate-poor ground from Martian and planetary surfaces.

**References:**

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**LONG-TERM PERSISTENCE OF MICROORGANISMS IN GEOLOGICAL MATERIALS: LAZARUS, RIP VAN WINKLE, AND THE WALKING DEAD.** P.J. Boston[1,2], M.N. Spilde[3], D.E.Northup[4], & C. McMillan[5]
[1]EES Dept. New Mexico Institute of Mining & Technology, 801 Leroy Place, Socorro, New Mexico 87801; pboston@nmt.edu, [2]National Cave and Karst Research Institute, 1400 Commerce Dr., Carlsbad, NM 88220; [3]Institute of Meteoritics, Univ. New Mexico, Albuquerque, NM 87131; [4]Biology Dept. Univ. New Mexico, Albuquerque, NM 87131; [5]Northern Arizona University, Flagstaff, AZ 86011.

**Introduction:** Many have speculated about the potential for very long term survival of microorganisms [1] and some investigators have reported such finds in halite even as early as the 1960's [2,3]. Besides the curiosity value of such reports, if they are true such very long-term viability of microbes has significant implications for Earth evolution, for life on other planets, and perhaps for the plausibility of microbial swapping from one planet to another, or even panspermia.

**Lazarus Taxa in Microorganisms:** Concepts in macroscopic evolution that pertain to very long lived "survivor" taxa that seem to disappear from the fossil record only to reappear in far distant future geological epochs [e.g. 4] may be simply a preservation issue with these organisms, but in the microbial world, we suggest that such "Lazarus Taxa" may actually be disappearing from the surface biota for geologically significant periods of time, surviving in the subsurface, only to be reintroduced to the aboveground biosphere by crustal geological processes (uplift, orogeny, cavern formation, faulting, canyon incision, etc.). If such genomic storage occurs in the subsurface, it may provide an additional means of evolutionary adaptation to changing environments aboveground, and similar mechanisms might enable quiescent survival of organisms during super-annual climatic cycles on planets like Mars.

**Rip Van Winkle Microorganisms:** Selenite (crystalline gypsum) crystals of extraordinary size have been discovered in cave chambers intersected by mining activity at the Naica Mine (Industries Peñoles) in Chihuahua, Mexico [5]. Within the crystals, inclusions are numerous. They often contain solid materials, fluids, and sometimes gas. In addition, highly colored red, orange, and black deposits on chamber walls appear to have surface morphologies suggestive of microbial biofabrics (Figure 1). The cave chambers are associated with an active hydrothermal base metal ore deposit and exhibit temperatures presently ranging from ~ 40-60°C. The chambers have been drained of hydrothermal water during mining of zinc, lead, silver and minor copper, thus allowing access to the chambers about a decade ago. During Feb. 2008 and Dec. 2009, we collected fluid and solid samples for direct microscopic inspection (Figure 2), bulk chemistry, live culture, and DNA analysis. Based on our results, we estimate that organisms that we have recovered from inclusions may have been trapped within their crystalline time capsules from between 10,000 to 100,000 years. The range of time depends upon the exact depth in the crystal that was sampled and which age estimation we accept for the crystals of the three studies that have been published to date. U/Th dates published are subject to uncertainties in the location of broken crystal sampled for the analysis. However, crystal growth experiments in the mine produced calculated ages of ~400 ky for the large crystals [6]. The deepest fluid inclusion that we sampled was at a crystal depth of approximately 3-5 cm which yields approximate ages of 30-50 ky since entombment. This range is based on growth rates ranging from 0.5 - 1.45 mm per 1 ky [7].

Molecular data from the fluid inclusions, and a variety of wall microbial/mineral deposits shows a plethora of strains that appear to be entirely novel, but whose closest relatives (at the 90% similarty range) include organisms from tantalizing environments around the world including volcanic soils, other caves in different hemispheres, and many that have been identified from a variety of unusual and extreme chemical circumstances.

**Walking Dead Microorganisms:** The experience of entombment in fluid inclusions, sediments, or other geological materials cannot be an easy one to survive. Extreme toughness in the face of environmental insults appears to be common in extremophiles, and quite noticeable in many subsurface strains that we have studied. Other recent experiments within our group that help to illustrate this amazing survivability have involved organisms that are making their living metabolizing copper sulfides, an interesting story in itself, but what we recount here of interest is the regrowth of organisms after being subjected to preparation and repeated examination with scanning electron microscopy (SEM) and electron microprobe techniques. Samples are air dried, dried in a vacuum oven at 100°C, vacuum-coated with Au/Pd coatings to render them electron dense, and then repeatedly exposed to high voltage electron beams for imaging, and stored between analyses in a dessicator. The copper sulfide microbial communities have regrown on the sample stubs, punching up through the Au/Pd coatings to produce the same brown fuzz that originally attracted us to study the copper sulfides in the first place! These organisms are tough little buckaroos, and perhaps a

model of good candidates for surviving geological entombment, long term burial in sediments, or even carried along as bioburden in spacecraft.

**Caveats and Concerns:** The primary caveats with claims of live microbial antiquity (or claims of extractable DNA) center around two issues. The first issue is potetial contamination of specimens with live environmental organisms or biomolecules that would be expected in a given environment. The second concern is geological and geochemical processes that may have compromised the original materials over geological time and overprinted them with organisms that could have been introduced into a system long after it's claimed age. Although one can never completely rule out contamination, the taxonomic affinities and variety of organisms identified by molecular and culturing techniques can provide some confidence that at least contamination is unlikely when large numbers of extremophiles are found. Addressing the second issue of geological alteration can be much trickier, requiring a plausibility argument based on the apparent pristine nature of the mineralogical and petrographic setting and a thorough discussion of all possible confounding geochemical processes that may have occurred in that setting over time. We now believe that our hot crystal communities, and the communities living in wall material in the Naica caverns both show that we are looking at Naica indigenes, some of which have apparently been off the social circle for quite some time! If we are correct, then this adds another geological storage possibility (crystalline gypsum) to the small list of materials in which long term microbial survival has been reported (ice and halite).

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*Figure 1: Biotextural appearance of red wall material in Cueva de Los Cristales, Naica, MX.*

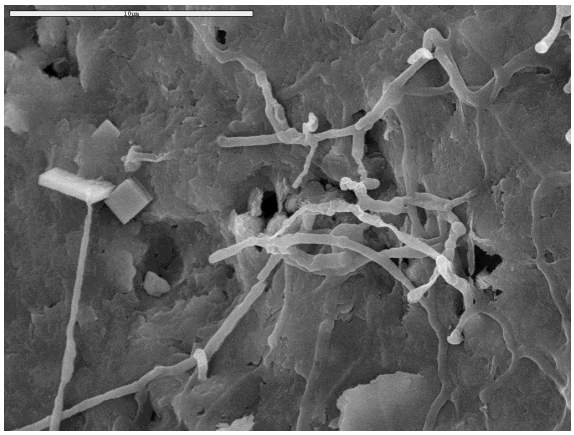

*Figure 2: Filamentous microbial forms protruding from iron oxide and clay coated cave chamber walls. These organisms are still very much alive in the walls (although presumably NOT on this SEM specimen stub…but you never know….see below).*

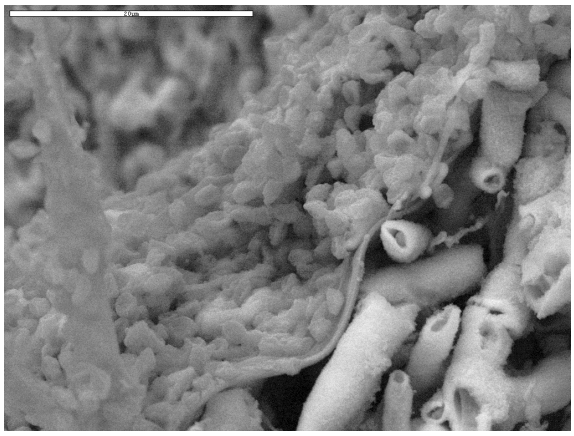

*Figure 3: Survivors of SEM preparation, small cells on left are bacteria-sized, broken hyphae on right are in the size range of fungi.*

**EPR STUDY OF PALEOGENE TO PRECAMBRIAN CHERTS: REASSESSING THE USE OF EPR FOR THE DETECTION OF CONTAMINATIONS IN VERY OLD CARBONACEOUS MATTER.** M. Bourbin[1], S. Derenne[1], D. Gourier[2], Y. Le Du[2] and F. Westall[3], [1]Bioemco, Paris, France (corresponding author mathilde.bourbin@upmc fr), [2]LCMCP, Paris, France, [3]CBM, Orléans, France.

The most ancient Life traces, as old as about 3.5 Gyr are recorded in siliceous sedimentary structures (cherts). If the mineral matrix may be accurately dated, using the U-Pb isotope scale notably, the dating of the organic matter (OM) embedded in the mineral matrix remains a difficult task. Indeed, OM may be more recent than the mineral matrix, due to various contamination processes (weathering, colonization by endolithic bacteria, anthropogenic contamination...). It is therefore of utmost interest to find syngeneity markers, establishing that the organic matter does not result from any contamination process and is of the same age than the mineral matrix.

Electron Paramagnetic Resonance (EPR) spectroscopy is a high sensitivity technique for the study of organic radicals in mature geological samples containing organic matter (coals, cherts, flints, meteorites…). The evolution of the shape of the EPR line was recently proposed as a proxy for syngeneity in cherts having a metamorphism level lower or equal to greenschist [1]. The EPR line namely evolved from a gaussian (for recent samples) to a lorentzian shape (for ca. 2Gyr old samples), ultimately reaching a "stretched-lorentzian" shape (for ca. 3.5Gyr old samples). This evolution reflects the change from a 3D to a 2D distribution of the radicals in the organic matter of the chert.

In order to test the reliability of this proxy, a wide set of chert samples was studied, ranging from the Paleogene to the Precambrian, and showing various metamorphic grades. EPR line shape of all samples was in agreement with chert age, and the proxy was established as statistically robust.

Importantly, EPR study of chert samples having same age but various metamorphism grades (but still lower or equal to greenschist) shows that metamorphism does not rule the evolution of the EPR line shape.

Moreover, to test the resolution of this syngeneity marker, artificially contaminated material was studied, revealing that EPR may detect contamination with a good resolution in time. A methodology for contamination detection is therefore proposed.

This study enhances that EPR is a powerful tool for the study of the OM in very ancient cherts, and that it can be extended to further exobiology studies.

**References:**

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**AN APPROACH FOR COLLECTION, CONCENTRATION, AND ISOLATION OF DNA FROM TERRESTRIAL AND EXO-PLANETARY SOILS USING SCODAPHORESIS.** C. Bradburne[1], C. Neish[1], C. Robinson[2], S. Kinahan[1], J. Proescher[1], J. Maydan[3], A. Marziali[3], and J. DiRuggiero[2].[1] The Johns Hopkins University Applied Physics Laboratory, Laurel, MD [2] The Johns Hopkins University, Department of Biology, Baltimore, MD. [3] Boreal Genomics, Los Altos, CA.

**Introduction:** Isolation of DNA from the genomes of soil-dwelling microbes is a difficult problem that requires extensive hands-on laboratory manipulation, as well as chemical and physical extraction techniques. SCODAphoresis is a new, enabling technology developed by Boreal Genomics (Vancouver, CA) which shows promise in this field, has few to no moving parts, and is amenable to automation. We are implementing this technique to isolate DNA from soils of astrobiological significance [1], with eventual development targeted for an automated, *in-situ*, and pressurized instrument on a Mars rover. In this presentation, we will show a comparative analysis of SCODAphoresis to other DNA extraction techniques for Mars-analogue, low-biomass soils.

*Synchronous Coefficient of Drag Analysis (SCODA).* SCODA is a novel technique for the concentration and purification of nucleic acids, developed by Boreal Genomics (Vancouver, CA). The movement of nucleic acids through an electrophoretic gel is non-linear in the presence of increasing charge, allowing a separation parameter. Using rotating electric fields, molecules can be driven in periodic motion that allows them to be focused, and DNA molecules are ultimately driven slightly toward the center relative to their starting location after each field rotation cycle. Contaminants, which exhibit linear motion in an electrophoretic gel, are driven in circular orbits, and so do not co-purify with nucleic acids. The resulting DNA is concentrated and free from normal soil inhibitors such as humic acid, which typically co-purify with DNA [2].

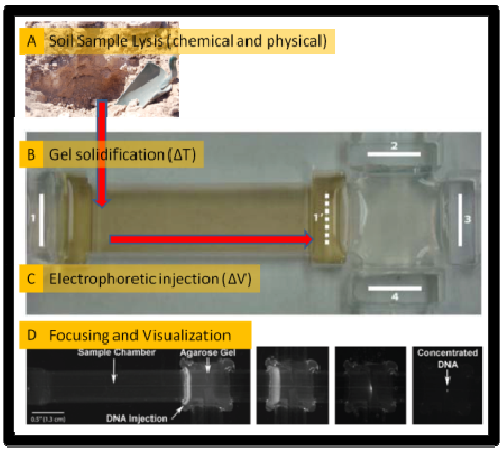


**Figure 1**: A schematic showing the sample preparation process for DNA focusing.

*The sample preparation process.* In Figure 1, soil samples (A) are extracted and placed into an un-solidified gel matrix containing components for chemical and physical lysis. A flow through Centricon or similar filter is used to reduce salinity of the lysed sample, and to provide a rough first removal of contaminants. Sample/gel matrix (B) is solidified by manipulating temperature. Sample in solid gel matrix is then injected into the focusing chamber (C) by applying an electric field in one direction. Following injection, DNA in the sample is focused (D) by application of rotating electric fields and collected in the center. Here, DNA bound to a fluorescent indicator present within the gel is visualized migrating through the gel to the central collection point (red arrow). For detailed physical equations describing the concept of SCODA phoresis, please see [2].

*Comparative analysis of extraction techniques.* DNA extractions, yields, and qualitative metrics are compared for material from the Bea Hill (BEA), Kevin Garden (KEV), and Andrew Garden (AND) locations in the hyper arid core of the Atacama desert in Chile, and from the University Valley (UV) and Pearse Valleys in Antarctica. These soils are all extremely challenging for genomic DNA extraction, due to oxidizing conditions, high salinity, and extremely low microbial load. We demonstrate a 10-fold or more increase of DNA recovery from these samples, and describe the communities herein.

The application of the SCODAphoresis technology to this problem is a first step in developing a future DNA collection, concentration, and detection capability on a future lander/rover. The instrument and process should be very amenable to development of an automated, *in situ* instrument that can serve as a DNA collection, concentration, and detection capability on Mars. In the context of Mars sample return, a SCODA-based instrument may prove ideal for characterization of the landing site, and final selection of the sampling area.

**References:**

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**Sample Integrity for the Proposed Mars Sample Return Campaign.** C. J. Budney, Jet Propulsion Laboratory / California Institute of Technology, 4800 Oak Grove Dr., Pasadena, CA 91109-8099, Charles.J.Budney@jpl.nasa.gov.

**Introduction:** "Sample integrity" has been defined as preserving the scientific value on the samples returned during the proposed Mars Sample Return (MSR). Sample integrity includes contamination control, sample selection, sample documentation, sample preservation, and any other items needed to make sure the samples have the highest science value.

It is important to note, however, that sample integrity needs to be performed within the constraints of the mission, so the potential requirements are often traded with the engineering capabilities of the mission to come up with a reasonable and implementable requirement. This paper will discuss the current thinking as to the sample integrity capabilities for the proposed first MSR.

**Previous work:** Considerable previous work has been done for sample preservation requirements of samples to be returned from Mars. The philosophy of sample integrity is well described in Gooding [1] and Neal [2]. However, these mission concepts had different constraints than the current set of missions being proposed. Therefore, they are not directly applicable to the current MSR campaign concept, but they provide a useful basis for comparison. The differences from these previous studies and the current approach, along with the reasons for these differences, will be discussed.

**Current Science Goals:** The Mars Exploration Program Analysis Group (MEPAG) End-to-End International Science Assessment Group (E2E-iSAG) [3] has developed proposed science objectives for MSR. The E2E-iSAG extensively discussed the sample types that would be needed to achieve the science objectives. Returned samples would include sedimentary and igneous rock cores, regolith, and atmospheric gas.

**Sample integrity:** Based on the E2E-iSAG objectives, to meet the science goals with the samples the E2E-iSAG led to certain requirements on the mission elements for collecting and protecting the samples. These are discussed briefly in the sections below.

*Size of the sample set.* The E2E-iSAG derived the needed mass of the samples based on expected analysis and curation on Earth. How this would be translated into a volume will be discussed.

*Sample encapsulation.* Each sample would be individually stored in a sealed Sample Capsule. Derivation of requirements for the seals will be discussed.

*Temperature control.* Temperature requirements would be needed to protect the samples from alteration. However, the cache would be passively thermal controlled and left on the surface for a decade. Implications for the thermal environments will be discussed.

*Magnetic fields and Radiation.* Potential needs for these requirements will be discussed.

*Physical integrity of the samples.* Sedimentary rock cores would contain significant information in the geologic structure preserved in the cores. Preventing the cores from shattering would be important. Approaches to this will be discussed.

*Inorganic contamination.* The Apollo experience has shown us that preventing inorganic contamination of the samples would be important for preserving the validity of scientific measurements. Some potential approaches for MSR will be discussed.

*Organic contamination.* One of the key scientific objectives would be looking for evidence of current life in the samples. Organic materials may be a key to determining the presence of life. Therefore, the requirements for limits on organic contamination would likely be considerable more stringent than for a previous Mars missions. However, the Mars Science Laboratory experience showed that there are certain limits on cleaning and preventing recontamination that may have significant implications for the science that could be done with the samples.

*Test materials and verification.* The materials used to test the sampling system would be important for ensuring the sampling rover could collect and preserve the samples.

**Conclusion:** Preserving the scientific value of the samples to be collected by MSR would be challenging, but approaches exist to return scientifically valuable samples. The ultimate approach would depend on a compromise between the scientific desires of the Mars community and the ability of the engineers to implement those desires. Discussion based on this presentation would help guide the development of the requirements for the proposed Mars Sample Return campaign.

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**SERS INVESTIGATION OF NUCLEOBASES DEPOSITED ON EVAPORITE MINERALS: A TEST CASE FOR ANALOGUE MARS REGOLITH.** S.Caporali[1,2], V.Moggi-Cecchi[1], M. Pagliai[2], G.Pratesi[3], V. Schettino[2,4], [1]Museo di Scienze Planetarie, Provincia di Prato, Via Galcianese, 20/h, I-59100 Prato, Italy, [2]Dipartimento Chimica, Università di Firenze, Via della Lastruccia, 3, I-50019, Sesto Fiorentino, Italy, e-mail: stefano.caporali@unifi.it, [3]Dipartimento di Scienze della Terra, Università di Firenze, Via La Pira, 4, I-50121, Florence, Italy, [4]European Laboratory for Non-Linear Spectroscopy (LENS) Università di Firenze, Via Carrara 1, I-50019, Sesto Fiorentino, Italy.

**Introduction:** Traditionally, Micro-Raman spectroscopy is one of the primary analytical techniques for the detection of minerals and organic molecules in the laboratory. However, in owe of its versatility and capability to interrogate samples without preliminary purification or concentration treatments, it is one of the frontrunners for the next generation of in situ instrument designed to explore Solar System bodies. In particular the Raman capability to unambiguously detect organic molecules and biomarkers might result of great importance to understand if and how primitive life generates in extraterrestrial environments (e.g. Mars). The technique's analytical sensitivity towards organic molecules can be further enhanced by means of the interaction with coin metals nanoparticles [1]. This approach, named Surface Enhanced Raman Scattering (SERS) has proved to be able to detect traces amount of nucleobases adsorbed on Terrestrial [2] or Martian [3, 4] substrates. Even if these studies have shown the suitability of SERS to detect tiny amounts of biomarkers in magmatic rocks, other substrates such as evaporites should result more prone to host biomolecules. According to orbital and rover observations [5, 6] as well as the presence of tiny amounts of evaporitic minerals in nakhlites (Martian meteorites) [7], Martian evaporites are considered to be constituted by carbonates, sulphates and clay minerals. In the present study we focused on the identification of nucleobases on Martian analogue material in order to test the SERS response on such kind of mineral substrates.

**Results and Discussion:** Two different nucleobases (adenine and hypoxanthine, Figure 1) were deposited on crushed samples made of carbonates (calcite, dolomite, magnesite and siderite) and sulfates (anhydrite and gypsum).

Then the Raman spectra were collected under air at room temperature on every single mineral phase. Without depositing Ag nanoparticles (Ag-nps) only the bands attributable to the substrate were observed (Figure 2).

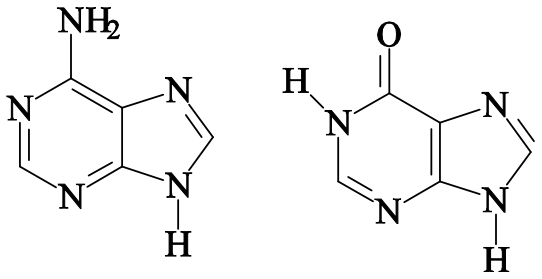


Figure 1: Structure of adenine (left) and hypoxanthine (right).

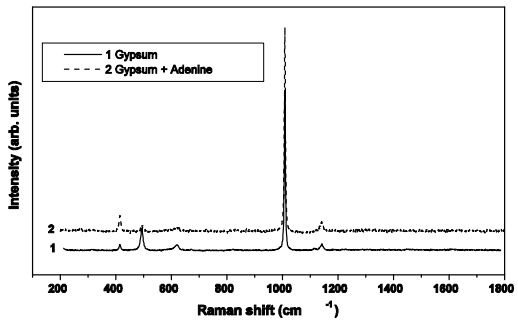


Figure 2: Raman spectra collected on a single grain of gypsum after (curve 1) and before (curve 2) the addition of the adenine dilute solution. Both spectra are dominated by the vibration bands attributable to gypsum. No adenine bands are detectable.

The addition of Ag-nps dramatically changes the appearance of these spectra. Alongside the broad band at 230-250 $cm^{-1}$ related with the Ag-nps and assigned to the Ag-Cl and Ag-N stretching modes, the Raman modes of adenine and hypoxanthine become clearly detectable (Figure 3). Apart from a large series of unresolved peaks in the spectral region between 1200 and 1700 $cm^{-1}$, attributable to overlapping vibrational modes present in both the molecules, intense and characteristic bands in the range between 700 and 750 $cm^{-1}$ are observed. The presence of the 734 $cm^{-1}$ peak is univocally assigned to the adenine breathing mode [8] while the two peaks at 726 and 743 $cm^{-1}$ are

assigned to the hypoxanthine breathing mode in neutral and anionic forms, respectively [9].

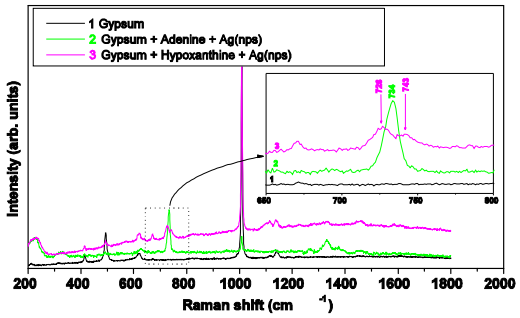


Figure 3: Raman spectra collected on a single grain of gypsum before (black line) and after the addition of adenine (green line) and hypoxanthine (magenta line) and Ag-nps. The inset shows a detail of the spectra in the 650-800 $cm^{-1}$ range.

The approximate quantity of sample responsible for SERS signal can be estimated assuming an uniform distribution of the nucleobases on a wetted surface area of ~20 $mm^2$ and a laser spot of ~3 $\mu m^2$. In such conditions the amount of sample involved in the SERS spectra is at level of $10^{-12}$-$10^{-13}$ g. Furthermore, among the tested substrates very slightly frequency shifts were observed [10], allowing to consider the peak at 734 and the two peaks at 726 and 743 $cm^{-1}$ as reliable markers for the identification of adenine and hypoxanthine, respectively.

**Conclusions and Perspectives:** Experimental evidence of the capability of SERS technique to detect traces of adenine and hypoxanthine deposited as dilute solution on evaporite minerals was provided. The Raman bands of these nucleobases were enhanced by SERS effect allowing their identification as small traces (about $10^{-12}$-$10^{-13}$ g). The results here displayed suggest the adoption of SERS as analytical procedures for *in situ* investigation on Mars. This only requires for Raman spectrometer adopted for the ESA ExoMars mission the most appropriated wavelength laser source and an automatic Ag hydrosol sprayer.

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This work has also been financed by MIUR-PRIN 2008 "Primitive Extraterrestrial Material as clues to the origin and evolution of the Early Solar System".

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**NUCLEIC-ACID SEQUENCING FOR LIFE DETECTION AND CHARACTERIZATION.** C. E. Carr[1*], C. S. Lui[1], H. Rowedder[2-3], M. T. Zuber[1] and G. Ruvkun[2-3], [1]Massachusetts Institute of Technology, Department of Earth, Atmospheric and Planetary Sciences, [2]Massachusetts General Hospital, Department of Molecular Biology, [3]Harvard Medical School, Department of Genetics. [*]Correspondence: chrisc@mit.edu

**Introduction:** Life on Mars, if it exists, may share a common ancestry with life on Earth due to meteoritic transfer of microbes between the planets [1, 2]. Recent discoveries of nucleic acids or their precursors within meteorites [3, 4] and in interstellar space [5] could steer the development of life towards these biomolecules; thus, one may want to search for RNA- or DNA-based life in habitable zones even outside the context of meteoritic exchange, such as in the probable liquid water oceans on Europa and Enceladus [6, 7]. We are building an instrument, the Search for Extraterrestrial Genomes (SETG)[8], to search for life via nucleic-acid sequencing in-situ on Mars.

Here we first discuss the detection limits of nucleic acid sequencing and the benefits of a metagenomic sequencing approach. We also propose a specific technology for in-situ sequencing that is small, robust, relies on hydrogen ion sensing instead of optics, and allows massively parallel sequencing. Initial sequencing results suggest it may be possible to generate enough reads to determine whether Earth and Mars organisms have a common ancestry and whether this split occurred before fixation of the genetic code, and to use any similarity in derived protein sequences to study how Mars organisms make their living. Finally, we discuss the relevance of in-situ sequencing to missions like Mars Sample Return (MSR).

**Detection approach:** A typical nucleic acid detection strategy involves extraction of nucleic acids while rejecting contaminants, followed by amplification via polymerase chain reaction (PCR), which amplifies DNA between two known regions (e.g. between highly conserved regions within the ribosomal 16S gene). Sequencing provides information on the (often unknown) region between the known regions. Because PCR and related amplification approaches such as isothermal whole genome amplification [9] can achieve single molecule detection, the detection limit is typically determined by limited yield during nucleotide extraction, with inhibitors playing a strong role in particularly vexing samples. Few studies have characterized absolute yield of DNA extraction, but the physical lysis (e.g. bead beating) required to break tough cell membranes (spores) achieves higher yields at the cost of increased DNA fragmentation.

**Detection limits for specific gene targets:** One experiment [10] found a 37% yield by bead-beating of filamentous fungi-spiked soils with median DNA size 0.5 /* 2 kb (e.g. log(size) has standard deviation of ~log(2)). Here, a 1kb region of the ribosomal 16S gene, assuming one copy per 2 megabase (Mb) genome, would be intact ~13% of the time (based on 1000 in-silico trials of assigning a target region to a random genome location, randomly sampling fragment lengths along that genome, checking for breaks within the target region). Thus, one viable DNA molecule might be expected for every ~20 cells at 37% yield. For fragment sizes much greater than the target region, the ideal detection limit is approximately equal to the yield (e.g. 10 /* 2 kb gives 93% intact, with estimated detection limit of 34% for a yield of 37%). This size distribution is typical of kits for isolation of DNA from soil, e.g. MoBio PowerSoil; here absolute yield dominates fragmentation in contrast to degraded (ancient DNA) samples, which can have median sizes of 100 bases or below. In lean samples, yields typically drop precipitously; however, an electrophoresis-related approach called synchronous coefficient of drag alteration (SCODA)[11] maintains >60% yield down to zeptomolar concentrations, while achieving ~$10^3$ better contaminant rejection than other approaches. Cartridge based detection systems manage to achieve detection limits down to 10 CFUs in some cases [12, 13]. Balancing quality and yield, a detection limit of perhaps $10^2$ viable cells may be feasible in-situ. A typical *E. coli* cell weighs 1 pg; at a density of $10^2$ cells/gram of soil, the cell mass is 100 parts per trillion (ppt). If 30% of the cell mass is organic material, an equivalent organics detector would need 30 ppt sensitivity.

**Metagenomic approaches:** Metagenomic sequencing [14], or sequencing any DNA molecule, should yield higher sensitivity that specific target-based approaches: First, metagenomic approaches do not depend upon extant organisms sharing specific conserved regions (like those within the ribosome). Second, highly sheared DNA can be utilized, allowing for sample preparation approaches that emphasize yield and achieve even better sensitivity than a specific gene target approach. An additional benefit is that metagenomics permits broad functional characterization of a microbial community through comparative sequence analysis in nucleotide and protein space. However, this analysis depends upon massively parallel sequencing. Specific informational sequences may be rare; for example, ribosomal sequences could be expected to represent 0.1% of metagenomic sequences.

**RNA-seq:** RNA can be reverse-transcribed to DNA and then sequenced. While RNA is easily degraded and thus not expected to be found except in viable organisms, RNA-seq can enhance the sensitivity of detecting ribosomal sequences; rRNA may represent up to 95% of total RNA in bacteria (e.g. ~10000 ribosomes in growing *E. coli*). rRNA count can be substantial even in slow growing organisms: The archaeal Richmond Mine acidophilic nanoorganisms (ARMAN) have ~1 Mb genomes and ~92 ribosomes in a 0.03 $um^3$ cell [15]. Thus, by performing metagenomic sequencing of RNA we can directly target ribosomal sequences, measure gene expression of DNA-based organisms, and detect a possible RNA world.

**In-situ sequencing:** Few high-throughput sequencing approaches [16] are compatible with in-situ sequencing due to their size and complexity. One exception is the Personal Genome Machine (PGM) from Ion Torrent [17], for which the key element is a 2.5 cm x 2.5 cm sequencing chip. Here, adaptors are ligated to fragmented DNA to produce library molecules, which are emulsion-PCR amplified at the single molecule level on beads. These beads are then loaded into the chip to achieve an ideal one bead per well. When nucleotides flow over a well and are incorporated, hydrogen ions are released, which are detected by an ISFET as a pulse of voltage, eliminating the need for optical detection. We sequenced *E. coli* DH10B (**Fig. 1**, 314 chip, 1.2 million wells), obtaining 52 Mb of data, over 40 Mb at 1% error. After ~30 runs to date, a typical good run gives 30-50 Mb of data (our record is over 80 Mb). Future sequencing chips with 10x more wells, combined with read lengths of 200-400 bp, may yield >1Gb/run. Thus, *if a single organism with moderate genome size dominated an environment on Mars, it may be possible to sequence its entire genome in-situ*. Follow up analysis may include nucleotide comparisons (placing an organism into the tree of life using ribosomal sequences) as well as searches for similarity in protein sequence space through translation of sequence reads. Even if Mars organisms have a different genetic code it may be possible to identify elements of this code through statistical analysis.

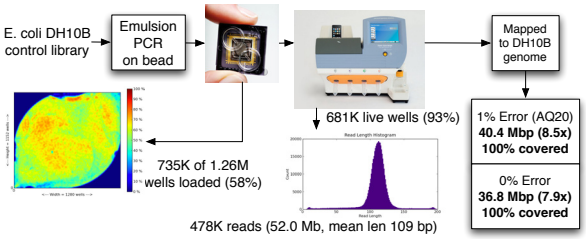


**Fig. 1 Sequencing *E. coli* on the Ion Torrent PGM.**

**Relevance to MSR:** Advances in sequencing may drive the specific approach used for future returned samples (for example, nanopore sequencing may enable robust single-molecule sequencing). However, there are several reasons to consider in-situ nucleic acid sequencing as part of MSR: First, a positive result would provide a strong incentive to complete the mission and return samples to Earth. Second, comparative sequence analysis of putative Martian organisms may help evaluate potential toxicity (or lack thereof). Third, such analysis could be used to prioritize what samples to return (with proper allocation of samples towards non-biological goals as well). Waiting to search for nucleic acids may also result in degradation due to exogenous or endogenous processes (e.g. cell lysis followed by degradation of RNA). Samples stored for years on the surface of Mars will receive large proton, heavy ion, and neutron doses; limited exposures conducted for SETG suggest DNA may survive such conditions for at least a couple years (data not shown).

**Conclusions:** Life detection through sequencing provides a highly sensitive and specific approach despite some obvious limits, e.g. inability to detect non-nucleotide based life or nucleotide-based life that uses incompatible nucleobases. It is now technologically feasible to pursue massively parallel sequencing in-situ, which could enhance the value of sample return missions. Given the possibility of shared ancestry between life on Earth and Mars, and the potential for RNA or DNA-based life elsewhere, searching for life *as we know it* is a critical part of any comprehensive life detection approach.

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**BIOSIGNATURES IN VESCICULAR BASALTS.** B. Cavalazzi [1], F. Westall[2], S.L. Cady [3], R. Barbieri[4], F. Foucher[2], N.J. Beukes[1], Department of Geology, University of Johannesburg, Johannesburg, South Africa (cavalazzib@uj.ac.za; nbeukes@uj.ac.za), [2]CNRS-Centre de Biophysique Moléculaire, Orléans, France (frances.westall@cnrs-orleans.fr; frederic.foucher@cnrs-orleans.fr), [3]Department of Geology, Portland State University, Portland, Oregon, US (cadys@pdx.edu), [4]Dipartimento di Scienze della Terra e Geologico-Ambientali, Università di Bologna, Bologna, Italy

**Introduction:** The search for traces of life on Mars is concentrated on sediments and alteration products that have been formed in the presence of water but volcanic rocks, in particular basalts, are the most common rock type on Mars. Basalts extruded under water or covered by water should not be ignored because it has recently been recognized that the glassy rinds of pillow basalts and vesicles and fractures in basaltic rocks represent ideal habitats for chemotrophic microorganisms [1-5]. They host a wide variety of euendoliths, chasmoliths, epiliths, and cryptoendoliths [e.g. 1, 3-5]. Importantly, such microorganisms can leave traces in the geological record [1-6].

In this presentation we describe microbial fossils in vesicular basalts [4].

**Fossil Life in vesicular pillow basalt:** A vesicular pillow basalt from the Ampére-Coral Patch Seamounts in the eastern North Atlantic was studied as a potential habitat of microbial life (Fig. 1) [4]. A variety of putative biogenic structures, such as filamentous and spherical microfossil-like structures, were detected in K-phillipsite–filled amygdules within the vesicles in chilled pillow basalt rinds. Several lines of evidence indicate that the microfossil-like structures in the pillow basalt are the fossilized remains of microorganisms. Possible biosignatures were investigated using a multianalytical approach (e.g. Raman microscopy, ESEM-EDX, CLSM).

**Astrobiological relevance of vesicular basalts:** This study documents a variety of evidence for past microbial life in a hitherto poorly investigated and underestimated microenvironment of significant relevance to Mars. Basaltic rocks that have been in prolonged contact with water should not be ignored as potential habitats and preservers of life. Although previous identifications of traces of life in the martian meteorite ALH84001 [7] are still debated, it is clear that such materials are excellent habitats for chemolithotrophic life forms.

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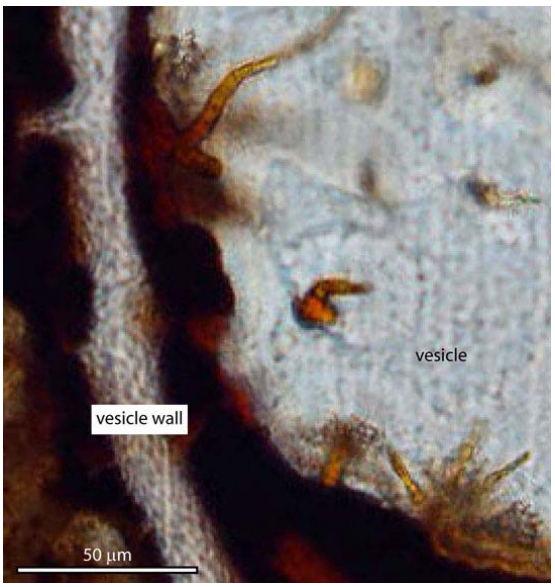


***Figure 1.*** *Transmitted-light photomicrograph of a petrographic thin section showing a phillipsite-filed vesicle in a pillow basalt from Ampère-Coral Patch Ridge Seamounts, North Atlantic. Note on the vesicle walls the presence of microfossils.*

**Aknowledgements:** BC would like to thank Eve Fisher and Christian Reinke from Analytical Facility-Spectrau, University of Johannesburg. We gratefully thank Le Studium, Institute for Advanced Studies, Region Centre, Orléans, France, and the NASA Exobiology Program and NASA Astrobiology Institute.

**INVESTIGATION OF THE SPATIAL RELATIONSHIPS OF BACTERIA ASSOCIATED WITH ROCK VARNISH.** A. C. Corcoran[1], D.R. Noguera[1] and K.R. Kuhlman[2], University of Wisconsin-Madison (3201 Engineering Hall, 1415 Engineering Drive, acrocoran@wisc.edu), [2]Affiliation for second author (402 Engineering Research Building, 1500 Eng. Research Bldg., Madison, WI).

**Introduction:** Understanding the characteristics of life in terrestial environments analogous to the martian environment influences the search for life on Mars [1-4]. Especially important is the search for life in niche environments that may harbor life in the arid and high UV radiated martian environment [1-4]. Rock varnish is a 10-500 μm thick coating with nanostratigraphic layering composed of approximately 70% clay minerals cemented together by 30% oxides and hydroxides of manganese and iron [3-4]. Rock Varnish forms in arid to hyperarid region and may exist as a UV shield for the organisms within it [1] and is a niche environment in areas where it is difficult for organisms to exist even in soil such as the Yungay reagion of the Atacama Desert [3]. Rock coatings that resemble varnish have been observed on Mars making this an environment worth investigating [1].

The goal of the research is to assess the spatial relationships between rock varnish from Martian analog environments and microbial communities that inhabit them. Fluorescence in Situ Hybridization (FISH) was used in order to determine these spatial relationships. FISH is the use of dye tagged oligonucleotide probes that target the the small or large subunit rRNA of an organism. The hybridization of this probe with the rRNA of organisms then yields information on the microbial ecology of samples in various environments [5]. The microbial ecology data available is the type of organism as well as its spatial relationship to other organisms as well as its immediate environment.

Due to low ribosomal content and high autofluouresnce, a variation of FISH with Catalyzed Reporter Deposition(CARDFISH) in order to vizualise cells in situ [6]. Using CARDFISH the spatial relationships of cells to the varnish can be established. Additionally identification of the community that inhabits this niche environment illustrates how terrestrial lifeforms survive in this niche envrionment.

**Sample Processing:** The field areas evaluated in this study were Cima Volcanic Field in the Mojave Desert California and an area near Darwin California. Samples were collected and shipped back to the University of Wisconsin-Madison for molecular analysis.

Varnish was ground off of the parent rock and then analyzed in several different ways. DNA was extracted form the Varnish and then the 16S or 18S rRNA was sequenced to determine the community composition of both samples. This rRNA data was then classfied to an 80% confidence level using the Ribosomal Database Project [7]. Also Phospholipid fatty acids (PLFA) analysis was performed in order to determine cell count and realtive community composition.

Community composition information from sequencing and PLFA anaylsis was then used to determine the rRNA target sites for probes for CARDFISH. This was performed on ground varnish in order to optimize a protocol for later CARDFISH on cross sections of the varnish.

CARDFISH replaces the fluorescent tag on the 5' end of the oligonucleotide probe used in traditional FISH with Horseradish Peroxidase(HRP). The HRP tag is reacted with fluorescently tagged tyramide. This process yields fluorescent data on the number and type of cell within the sample when analyzed by confocal microscopy.

**Results:** PLFA analysis yielded cell counts of $6.69\ E^{7}$ (cells/gram)to $1.54E^{8}$(cells/gram) indicating that a significant amount of life does exist in this niche environment. The 16S and 18S sequence data discoverend in this study represents a significant increase in the total known sequences from varnish environments. In the Ribosomal Database Project there are 167 sequences found in Rock Varnish studies. 141 16S seqeunces were discovered in varnish from this research. This is resprents a signficant expansion in 16S knowledge of Bacteria and Archaea and 18S knowledge for Eukaraya. The organisms found include Cyanobacteria, Alphaproteobacteria and Thermoprotei.

The CARDFISH results show several successful hybridizations and were able to get around much of the autofluoresnce that would typically plague this kind of research. Figure 1 is an image of a piece of rock varnish that has been hybridized with the bacteria targeting probes EUB338 I, II, III. This represents a breakthrough in terms of obtaining successful in situ results from rock varnish. A probe targeteing Archaea was also successfully hybrized in these samples to give an idea of microbial community structure.

The CARDFISH results from these images provide the community compostion and spatial relationships to help understand this terrestrial niche environment. The organisms that survive in rock varnish may yield clues as to which environments organisms would survive on Mars.

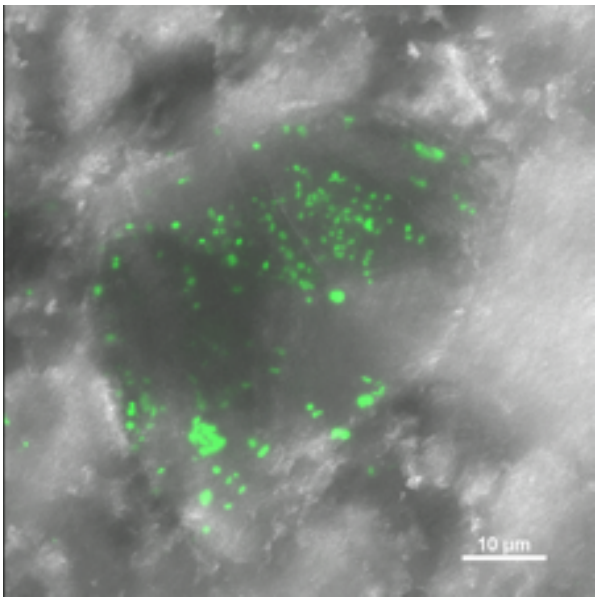


**Figure 1. Transmitted light and 488nm image of Rock Varnish from the Cima Volcanic field in the Mojave desert. Sample has been hybridized with**

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**Sterilization of Spacecraft Components by Laser Ablation and Plasma Generation.** C. B. Dreyer[1], J. R. Spear[1], K. L. Lynch[1], and A. J. Bauer[2], [1]Colorado School of Mines, Golden, CO 80401, cdreyer@mines.edu, [2]Applied Research Associates, 7921 Shaffer Parkway, Littleton, CO 80127.

**Introduction:** In the coming decades spacecraft will travel to destinations that may harbor evidence of past or present life. A Mars sample return mission will likely return samples to Earth from an area of Mars that will be thought to have been habitable in the ancient past. Such missions increase the need for new planetary protection (PP) methods. We are investigating the use of focused laser beams to ablate the surface of spacecraft surfaces and any contaminating materials. The method can be applied such that the ablated material is heated to a plasma, making it a LIBS (laser induced breakdown spectroscopy) approach. As with traditional LIBS, the plasma emission can be analyzed and used to determine if the ablated material is from the known spacecraft material or a foreign component. The method could be use in-transit or on the martian surface. It can also be applied to the exterior of sample return canisters and is amenable to other surface missions, such as a landing on one of the moons of Jupiter or Saturn.

**Sterilization with focused laser beams:** Focused laser beams have been used to kill microbes inside the mouth and in wounds [1] [2] using low irradiance laser beams (~1 W/cm$^2$). Ablation of most materials requires laser irradiance above a threshold on the order of tens of MW/cm$^2$ [3]. An irradiance level well above the ablation threshold will cause a plasma to form. A plasma that can be analyzed by the principles of LIBS requires irradiance on the order of 10 GW/cm$^2$ [4]. Laser irradiance below the ablation threshold can weaken cell membranes causing loss of membrane integrity. Oxidative species that destroy enzymes and DNA can also be generated by this plasma. High irradiance laser beams can sterilize surfaces by ionizing the entire surface and all contaminating materials. Under the right conditions the plasma emission generated by high irradiance beams can be used to determine the elemental composition of the ablated material.

**Laser Sterilization Concept:** Figure 1 shows a concept of how laser sterilization may function in practice. A robotic arm with several degrees of freedom is used to raster scan the laser across the surface of a part, i.e a sample container. The part may be held fixed or translated in concert with the robotic arm. Emission from a LIBS plasma is collected by a spectrometer mounted on the robotic arm, as shown, or placed off the RA and light collected by optical fiber. The particular means of implementing the laser ablation sterilization method will depend on several factors including the shape of the part and mission objectives.

The range of laser irradiance need to implement the laser sterilization method is likely to be from about 1 MW/cm$^2$ to 10 GW/cm$^2$, depending on whether the objective is sub-ablative sterilize, ablation of the surface without plasma or generation a LIBS plasma. The laser irradiance has important implications for setting the laser power consumption, laser spot size at the surface, raster scanning rate, and time for sterilization.

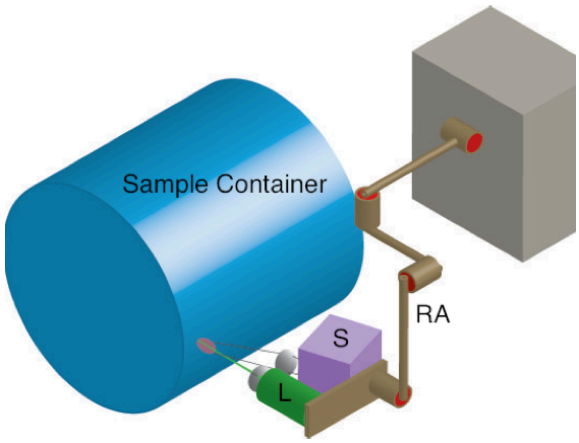


***Figure 1:*** *Schematic of the laser sterilization concept treating a large part. L: laser and focusing optics. S: miniature spectrometer. RA: Robotic arm. Cameras would also be used to position the RA (not shown).*

The rate at which the untreated surface is removed by ablation is:

$$\frac{\mathrm{d}a_u}{\mathrm{d}t} = -a_l f \frac{a_u}{A}$$

integration yields:

$$a_u = Ae^{-t/\tau_s}; \quad \tau_s = \frac{A}{a_l f}$$

Where $a_u$ is the total untreated surface area, $a_l$ is the area treated by the laser per laser shot, $A$ is the total area of the part, and $f$ is the laser repetition rate. The expression is an exponentially decreasing function with time constant $\tau_s$. Full surface treatment is approached after several times constants of treatment (99.3% of the surface is treated after 5τs).

In Table 1 the relationship between laser focal spot diameter, laser pulse energy, and laser repetition rate is shown for a fixed surface area of $1m^2$. Laser input power of 10W with 25% conversion to optical power is assumed as this a reasonable value for a spacecraft instrument. Laser irradiance is sufficient to produce a LIBS plasma on every laser pulse. These parameters yield a sterilization time constant $\tau_s$ = 22.2 hours for all spot diameters. A low repetition rate laser with a high pulse energy would be needed for focusing with a large spot diameter. A tightly focused laser beam would require very low pulse energy but very high repetition rate. A moderately focused beam (100 μm) would require a few mJ per pulse and a few KHz repetition rate, such a laser is common for diode pumped Nd:Yag and Nd:YLF lasers. At lower than 10 $GW/cm^2$ irradiance, either in sub-ablation sterilization or non-LIBS plasma forming ablation achieved by defocusing, sterilization could be achieved more rapidly with intermittent LIBS measurements to verify surface condition.

**Table 1:** Relationship between spot size and laser repetition rate at 10W laser power (25% efficiency), 10 $GW/cm^2$ irradiance, 2 ns pulse, total sterilization area $1m^2$. With these parameters $\tau_s$ is 22.2 hours.

| Spot diameter (μm) | Pulse Energy (mJ) | Repetition Rate (Hz) |
|---|---|---|
| 1500 | 353.4 | 7.1 |
| 1000 | 157.1 | 15.9 |
| 500 | 39.3 | 63.7 |
| 100 | 1.57 | 1591.5 |
| 50 | 0.393 | 6366.2 |
| 10 | 0.016 | 159154.9 |

LIBS plasma emission can be used to classify the type and amount of contaminants that were present before sterilization. LIBS spectra can be categorized according to known surface material, material of biological origin, material of mineralogical origin or unknown material. Plasma temperature decreases below 1 torr due to cooling by adiabatic expansion of the plasma expanding into vacuum, which causes a reduction in lifetime and emission intensity, however, emission is known to be observable and relatively constant below 0.1 torr ambient pressure [5] [6].

**Conclusions:** Laser sterilization of spacecraft components by ablation of the surface and contaminating material appears to be a plausible planetary protection method. The method has several advantages:

- Direct measurement of the sterilized surface. As the LIBS spectra of the man-made surface will be well known, deviations will be easily detected, and the contamination level determined.
- The surface and contaminating material are destroyed. The method can be operated to ionize the surface material. No known microbe can survive a direct hit by a high irradiance laser beam.
- The sterilization system can also double as an *in situ* instrument for scientific investigations.

The method could be useful in several potential PP mission scenarios such as:

- In-space sterilization of a Mars sample return canister. This scenario offers ample time for sterilization before returning samples to earth by performing sterilization in Mars orbit or on a Mars-earth trajectory.
- Sterilization of hardware on the Mars surface for manned and robotic missions. Hardware removed from a zone with high biological potential could be sterilized before entering a human habitation zone or a PP safe zone.
- Forward contamination, particularly missions in which in-space sterilization is needed to reduce the bioburden after launch, such as landed missions to the moons of Jupiter and Saturn.

**Future work:** Areas of future work are:

- How physical processes couple with microbe biological transport processes during sterilization. Atmospheric LIBS forms a shockwave that could induce transport of microbes and mineral dust near to the intense laser focus, while in a vacuum the shockwave is much reduced as it can only be formed from ablated material.
- The survivability of microbes due to a near miss of the ablation beam must be examined. Does a LIBS ablation pulse effectively sterilize a larger area than the ablated area?
- Operational and technical considerations: What is the best combination of laser focal spot size, pulse rate, and pulse energy? What scanning system is optimal? Can the method reach into crevices and into corners? Does the efficacy of the method vary with surface material? What is the surface finish of a laser sterilized part?

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**RECOVERY OF ORGANICS AND BIOMARKERS FROM MARS ANALOGUES AND METEORITES: EXTRACTION AND QUANTITATIVE ANALYTICAL CHALLENGES.** P. Ehrenfreund[1], R. Quinn[2], Z. Martins[3], S.O.L. Direito[4], B.H. Foing[4,5], M.J. Kotler[6],W.F.M. Röling[4]. [1]Space Policy Institute, Washington DC, USA (email: pehren@gwu.edu), [2]NASA Ames Research Center, Moffett Field, CA 94035, USA**,** [3]Dept. of Earth Science and Engineering, Imperial College London, London, UK, [4]Vrije University Amsterdam, NL, [5]ESTEC, SRE-S, Postbus 299, 2200 AG Noordwijk, NL, [6]Leiden Institute of Chemistry, Einsteinweg 55, 2333CC, Leiden, NL

**Introduction:** The analytical precision and accuracy obtainable in modern Earth-based laboratories exceeds that of any in-situ instrument onboard spacecraft. Therefore, a sample return mission to Mars has been identified as the highest priority for future Mars exploration. The Mars science community, in their inputs to MEPAG and the 2011 Planetary Decadal Survey, voiced that carefully selected samples from well-chosen sites would be the means to make the greatest progress in Mars planetary exploration and the search for life in the solar system. Compiled data from Mars orbiters and Mars rover surface operations help to define the best possible landing sites for future missions that will cache samples from regions that may harbor organic compounds (intrinsic or delivered). The landing sites for current and future missions target predominantly regions with a geological history facilitating the formation and preservation of organic molecules over long time scales. Therefore, environments that witness aqueous mineral processing, (e.g., phyllosilicate environments) are of particular interest. For future missions, the successful hunt for organic molecules and biomarkers will require consideration of several parameters including, deposition history and diagenesis, preservation potential, extractability and the instrument performance.

**Challenges:** The most sensitive techniques used to date to extract extraterrestrial organic matter from its host matrix use several extractions steps including water, solvents, heating cycles and chromatographic purifications. Extraterrestrial samples such as the Murchison CM2 meteorite contain amino acids at ppm concentrations; even 20 times larger concentrations of amino acids are recently found in Antarctic CR2 meteorites [1]. In comparison, concentrations of amino acids in samples from dry deserts such as Atacama are often devoid of recoverable amino acids or at the detection limit in Earth laboratories (< ppb concentrations). Tests in extreme environments on Earth have shown that even without restricted sample access, the recovery of organics in such materials is challenging [2]. Recent results from astrobiology field research have indicated that soil porosity and low clay-particle content seem to be correlated with extractability of organics and DNA. Even with access to analytical accuracy and sensitivity of Earth-based laboratory experiments that involve solvent extraction and amplification neither DNA nor amino acids could be detected in many samples [3,4]. In fact, clay material is known to strongly adsorb and bind organic molecules, often preventing extraction by even sophisticated laboratory methods.

Additionally, our results show dramatically low DNA recovery from clay-rich Utah desert samples that were spiked with a PCR fragment of the yeast *Saccharomyces cerevisiae* hexokinase1 (YHXK1) gene. Furthermore, the analysis performed on de serts samples has shown that organics (e.g. amino acids) and microorganisms have patchy distributions, with concentrations either above or below detection limits for samples collected within several meters proximity [5].

These issues not only complicate future *in situ* searches for biomarkers and organic compounds on Mars, but also the selection of samples for return to Earth. The complexity of searching for life traces on Mars reinforces the continuing need for ground truth studies to enable the successful detection and characterization of organics on Mars. Ground truth studies must include Mars analogue field research in extreme environments, laboratory investigations of biota and biomarker chemistry under Mars conditions (e.g., radiation, temperature, oxidation, racemization) as well as supporting analytical methods (e.g., optimizing extraction procedures that can release adsorbed biological compounds) We provide a comparison of results from recent analogue field tests in the Atacama and Utah desert and meteoritic sample analysis as well as a discussion of amino acid extraction and DNA binding on Mars analogue samples.

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**X-RAY COMPUTED MICROTOMOGRAPHY AND FLUORESCENCE MICROTOMOGRAPHY: NON-INVASIVE SCREENING TOOLS FOR RETURNED ROCK AND CORE SAMPLES FROM MARS AND OTHER SOLAR SYSTEM BODIES.** George J. Flynn, Dept of Physics, SUNY-Plattsburgh, 101 Broad St. Plattsburgh NY 12901 (George.flynn@plattsburgh.edu).

**Introduction:** NASA's Mars Exploration Program includes plans for a 2018 mission carrying a NASA rover that would collect and cache rock samples for potential delivery to Earth by a future mission. In addition, NASA's recently selected OSIRIS-Rex mission is expected to deliver to Earth rock fragments from the surface of an organic-rich asteroid "1999 RQ36" in 2023. In each case the samples are expected to be small (~ cm-size or less). A sensitive, non-invasive screeing of these rock samples to identify fluid inclusions, gas-filled inclusions, and possibly fossils is desirable to support the search for life. Fluids and gasses may contain important clues to prior conditions on Mars or RQ36 that constrain or identify the presence of life.

Unless the location of these inclusions is determined prior to cutting or breaking the rock the valuable fluids or gasses can be lost. The X-ray Computed MicroTomography (CMT) and Fluorescence MicroTomography (FMT) are sensitive, non-invasive screeing tools having high spatial resolution that are particularly well suited to the preliminary screening of these samples.

**Techniques:** We have performed CMT on meteorite samples [1] and FMT on interplanetary dust particles (IDPs) [2] with results that demonstrate the applicability of these techniques to the prescreening of extraterrestrial samples returned from Mars and other Solar System bodies.

*Computed MicroTomography (CMT).* We performed CMT analyses on the bending magnet beamline of the GeoSoilEnvironmentalCARS at the Advanced Photon Source (Argonne National Laboratory). This beamline provides x-rays ranging from 5 to ~100 keV. A Si (220) channel-cut monochrometer was used to provide a monochromatic beam, which illuminated the enture sample. X-ray transmission images were collected by viewing a YAG scintillation screen, located downstream from the sample, with a Princeton Instruments Pentamax CCD camera. We collected 360 transmission images in 0.5 degree steps covering 180 degrees of rotation.

Meteorite cores and small whole stones ranging from ~1/3 to 1 cm in diameter were analyzed. A 40 keV monochromatic x-ray beam, an energy sufficient to penetrate a 1 cm stone, was used. Each voxel in the reconstructed image was ~30 micrometers in each linear dimension. Sufficient contrast was obtained to identify cracks, high-Z inclusions, and vugs in an ~½ cm stone in ~45 minutes per sample. Improvements to the imaging system should provide at least 4 times better spatial resolution by the time of Mars of RQ36 sample delivery.

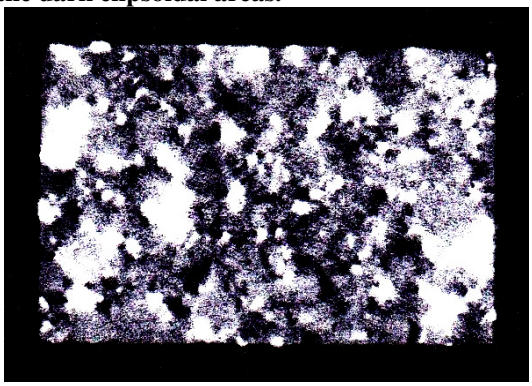
*Fluorescence MicroTomography (FMT).* We performed FMT analyses on the undulator beamline of the GeoSoilEnvironmentalCARS at the Advanced Photon Source (Argonne National Laboratory). The x-rays were focused to an ~3 micrometer spot using Kirkpatrick-Baez mirrors. The fluorescence tomography data were obtained by translating the particle, which was mounted on the end of a silica fiber with clean silicone oil, through the x-ray beam in 2 micrometer steps. At each position the fluorescence spectrum was collected with a solid state energy dispersive x-ray detector, which allows multiple elements to be detected simultaneously. The sample was then rotated by 0.5 degrees about the vertical axis and the line scan was repeated. The process was continued until the particle had been rotaed through 180 degrees, sampling a 2D plane through the particle. By successively translating the particle vertically by 2 micrometers and repeating the procedure at each height a full 3D fluorescence map of the particle was obtained.

One complication in this element-specific imaging technique is the escape depth of the fluorescent x-rays. In chondritic material the 1/e escape depths for the K-line fluorescence x-rays are ~0.2 micrometers for C, ~6 micrometers for Si, ~10 micrometers for S, ~30 micrometers for Ca, and ~100 micrometers for Fe. Thus, for the light elements the data reduction requires an iterative procedure in which successive element distributions and their appropriate absorption corrections are determined. In larger particles mapping the distriburttion of heavy trace elements that exhibit the same chemical behavior as certain light elements allows inference of the distribution of elements whose fluorescence x-ray are severely absorbed (e.g., Sr serves as a proxy for Ca and Se serves as a proxy for S).

**Results:** The results from our analyses of meteorites and interplanetary dust particles demonstrate the utility of CMT and FMT as non-invasive screening tools to search for evidence of life on Mars.

*Identification of Gas-Filled or Fluid Inclusions:* Minute amounts of Martian atmosphere trapped in inclusions in glass from the Martian meteorite ETTA 79001 provided evidence that the SNC meteorites were from Mars, and demonstrated that samples of Mars'

**Figure 1: CMT virtual slice through an ~1x1x2 cm block of the Mt. Tazerzite meteorite showing vugs as the dark elipsoidal areas.**



atmosphere are preserved in the rocks. More recently, the discovery of fluid inclusions in salt crystals in the Monahans and ZAG meteorites provided the opportunity to analyze liquid water from asteroids. The analysis of these fluids and atmospheric gas samples can have important implications for past life. For example, the presence of large amounts of oxygen in a planet's atmosphere has been proposed as a strong indicator of life [3].

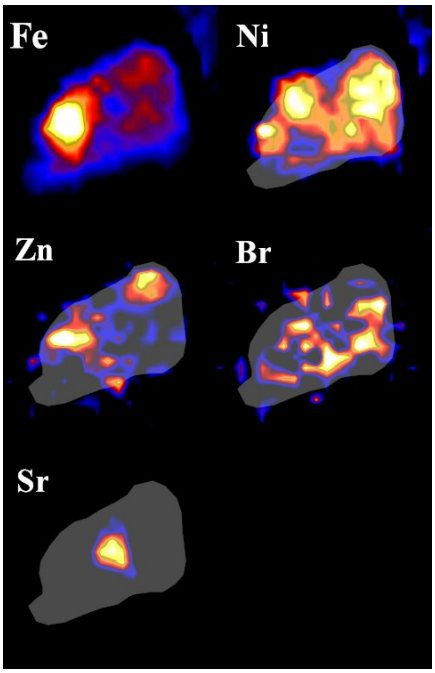
However, the analyses of trapped fluids or gases requires the identification of the subunit of the sample containing the fluid or gas before breaking the wall of the inclusion. While fluid- or gas-filled inclusions can sometimes be identified in thin section, if they are rare a large amount of sample can be consumed in this preparation.

CMT provides a quick, efficient tool to search for fluid or gas inclusions in centimeter-size samples. CMT images we obtained on the Mt. Tazerzite meteorite identified vugs as small as 2 voxels in size [1], as shown in Figure 1.

*Identification of Fossils:* If the density or average atomic weight of a fossil differs significantly from the host rock, CMT is an efficient tool for identifying and imaging fossils in rock samples. For example, propagation phase contrast X-ray synchrotron imaging was used to identify invisible fossil inclusions in fully opaque amber [4].

*Element mapping:* FMT provides a 3-D image of the distribution of elements in the sample, as shown in Figure 2 for an ~30 micometer interplanetary dust particle. If any fluid inclusions are identified by CMT, FMT can produce a preliminary elemental characterization of the heavier elements in the fluid, providing clues to its origin. FMT on fossils could provide better contrast between the fossil and the host rock as well as information on the composition and structure of the fossil.

**Figure 2: Fluorescence microtomogtaphy (FMT) images of the distributions of Fe, Ni, Zn, Br, and Sr (a proxy for S) in a virtual slice through the ~30 micrometer interplanetary dust particle L2036H19. The gray aera is the outline of the particle.**




**Conclusions:** CMT is a useful, non-invasive screening tool for cm-size samples, including samples collected by an initial Mars Sample Return mission and samples of the organic-rich asteroid 1999 RQ36. CMT is an efficicient, non-destructive technique to identify vugs, potentially containing fluids or gases from an earlier era on the parent body, as well as fossils in these samples. FMT, although limited by fluorescence absorption to smaller samples, ~200 micrometers, can provide elemental analyses of the fluids and fossils identified by CMT.

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**ABIOTIC CORROSION MICROTEXTURES IN VOLCANIC GLASS: REEVALUATION OF A PUTATIVE BIOSIGNATURE FOR EARTH AND MARS.** J. E. French[1] and D. F. Blake[2], [1]Department of Earth and Atmospheric Sciences, University of Alberta, 1-26 Earth Science Building, Edmonton, AB, Canada, T6E 2E3, jef@ualberta.ca, [2]NASA Ames Research Center, MS 239-4 Moffet Field, CA, USA, 94035-1000, David.F.Blake@nasa.gov.

**Introduction:** Understanding the impact of radiation damage on characteristic microtextures developed during the natural abiotic corrosion (palagonitization) of basaltic glass by seawater is critical to interpreting alteration microtextures in glasses on both Earth and Mars. Microscopic microbial trace fossils (biogenic etch features) are reported to be widespread in partially palagonitized submarine volcanic glasses on Earth [1]. However, alternative abiotic explanations for such conspicuously 'biogenic looking' microtextures in volcanic glass (i.e. grooves, tubular and granular textures) have recently been proposed [2]. It has been suggested that the presence of such microtextures in Martian basaltic glasses, should they be found, would constitute evidence for past microbial life on that planet [3], an hypothesis that should be critically reexamined.

Basaltic glass and palagonite are thought to be widespread on Mars [4], and there is abundant evidence for past action of liquid water on its surface [5]. In preparation for future Sample Return missions from Mars, it is imperative that we understand what constitutes a true microscopic morphological biomarker in terrestrial volcanic glass, and what alteration microtextures are abiotic in origin and simply 'look' biogenic to our eyes.

In the present study we highlight a rich diversity of microtextural corrosion features that occur in partially altered (palagonitized) basaltic glass pillow margins from Deep Sea Drilling Project (DSDP) Hole 418A, North Atlantic Ocean (Figs. 1-3). Although these various alteration microtextures look conspicuously like microbial trace fossils (e.g. microbial borings), we propose that they are all abiotic in origin, the result of preferential corrosion of radiation damage (alpha-recoil tracks and fission tracks) caused by radioactive decay of U and Th in the glass. Pressure solution fingering also seems to be an important compounding process in forming these complex networks of microscopic etch-tunnels at the glass-palagonite interface, caused by incremental increase of hydrostatic pressure (29 to 63 MPa) as the oceanic crust subsides under a deepening ocean with age. Features resulting from the abiotic (U-Th-Pb) radiogenic corrosion of basaltic glass by seawater appear to explain these and other putative 'tubular' and 'granular' microbial trace fossil microtextures, reported from a variety of environments including basaltic glass in the in situ oceanic crust, ophiolites, and greenstone belts dating back to ~3.5 Ga [1].

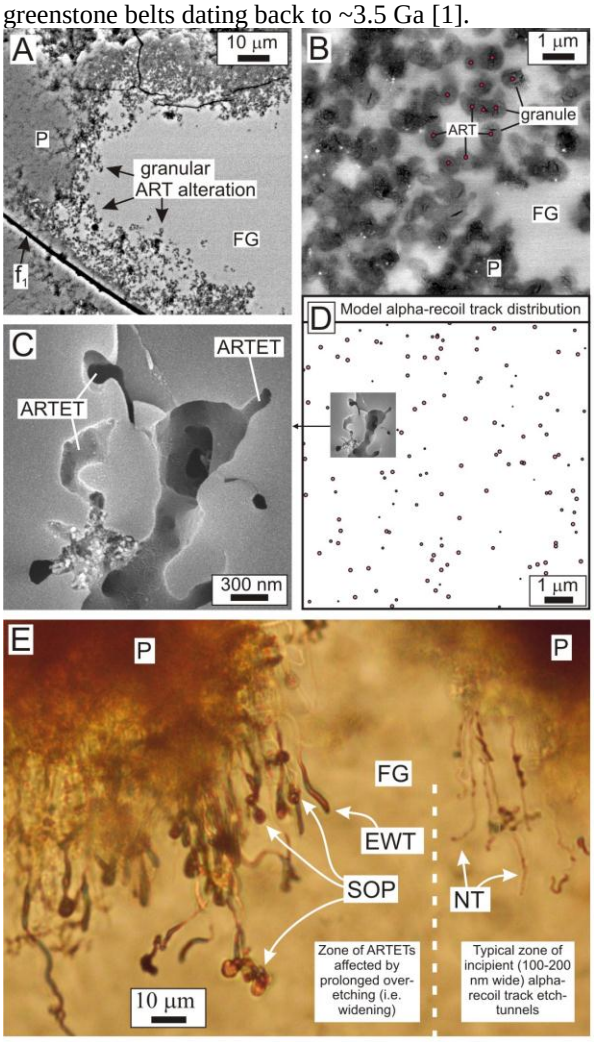


**Fig. 1.** A diverse suite of microtextures caused by abiotic corrosion of alpha-recoil track radiation damage in basaltic glass by seawater. **A**-**B**) Granular palagonite alteration caused by preferential palagonitization of alpha-recoil tracks (back-scattered electron images of a thin section). **C**) Alpha-recoil track etch-tunnels found at the glass-palagonite interface (secondary electron image of a freshly fractured surface). **D**) Calculated distribution of alpha-recoil tracks in DSDP-418A-75-3[120-123] basaltic glass (inset of **C** is shown at the same scale). Note the similarity in distribution of palagonite granules (**B**) with alpha-recoil tracks (**D**). **E**) Photomicrograph (uncrossed polars) of a thin section showing a diverse suite of etch-tunnel types related to incipient alpha-recoil track etch-tunnelling (right), including those which have been widened by prolonged over-etching (left). ART - alpha-recoil track; ARTET - alpha-recoil track etch-tunnel; EWT - elongate wide tunnels; $f_1$ - early fracture; FG - fresh basaltic glass; NT - nanotunnels; P - palagonite; SOP - string-of-pearls texture. (**A**-**B**) are from sample DSDP-418A-68-3[40-43]; (**C**-**E**) are from DSDP-418A-75-3[120-123].

**U-Th-Pb Radiogenic Corrosion Microtextures:** A comprehensive multidisciplinary study of two glassy pillow margins from DSDP 418A reveals the presence

of several distinctive abiotic corrosion microtextures at the glass-palagonite interface, including granular palagonite alpha-recoil track ("ART") alteration (Figs. 1A-B), ART etch-tunnels (Fig. 1C), and a diversity of wider etch-tunnel types formed by prolonged over-etching of incipient alpha-recoil track etch-tunnels, including elongate wide tunnels and string-of-pearls texture (Fig. 1E). Numerical modeling of alpha-recoil track damage in DSDP 418A basaltic glass based on U and Th concentrations in fresh glass measured by ICP-MS, indicates that the 108 million year old glass is riddled with radiation damage in the form of ~120 nm wide alpha-recoil tracks (Fig. 1D), which suggest a genetic relationship with nanoscopic 'tubular' etch-tunnels and 'granular' palagonite at the glass-palagonite interface (Fig. 1). These observations call into question the biogenicity of so-called 'tubular' etch-tunnels and 'granular' palagonite observed in worldwide samples of basaltic glass [1, 6].

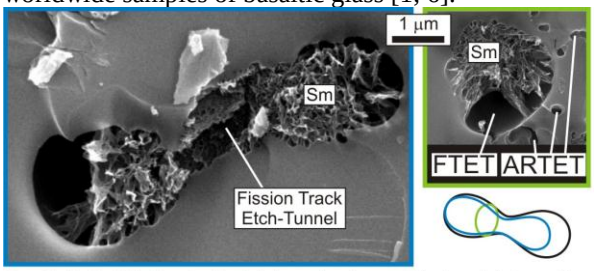


**Fig. 2.** Typical fission track etch-tunnels observed in the etch-tunnelling zone at the glass-palagonite interface in submarine basaltic glass. (secondary electron images of freshly fractured surface of sample DSDP-418A-75-3[120-123]). Typical ~8 μm long, peanut shape of FTETs shown at lower right. ARTET - alpha-recoil track etch-tunnel; FTET - fission track etch-tunnel; Sm - authigenic platy smectite.

Rare, larger (1-2 μm wide by ~8 μm long), peanut-shaped etch-chambers are also observed (Fig. 2) along with the smaller and more abundant nanoscopic tunnel variety (e.g. ARTETs in Figs. 1C, 2) at the glass-palagonite interface, and these larger etch-chambers are identified as naturally etched fission tracks (Fig. 2). These features might also be confused with microbial borings.

Naturally formed fission track etch-tunnels are always partially infilled with authigenic platy smectite showing dissolution/co-precipitation textures with glass (Fig. 2). The smaller, more abundant variety of nano-tunnels (alpha-recoil track etch-tunnels) are typically empty (Fig. 1C), although many contain authigenic imogolite $(OH)_3Al_2O_3SiOH$, which forms flexible 20 Å wide nanofilaments observable with high resolution scanning electron microscopy (Fig. 3) [7]. Imogolite filaments are identified as such by direct comparison with the known dimensions and morphology of typical imogolite filaments (superimposed blue/pink lines in Fig. 3), and considerations of the geological setting (imogolite is typically described as the initial weathering product of glassy volcanic ash [8]). It is important to note that these imogolite filaments (Fig. 3) might potentially be confused with the elongate filaments that can occur in desiccated exopolysaccharide mucus produced by bacteria in rocks [9], and they are also identical in size and form to filamentous strands of DNA imaged by atomic force microscopy [10] - biofilaments that coincidentally, are also exactly 20 Å wide.

Consequently, when evaluating corrosion microtextures in returned samples of Martian glass, nanofilaments within etch-tunnels should also be viewed as abiotic unless compelling evidence of biogenicity is presented.

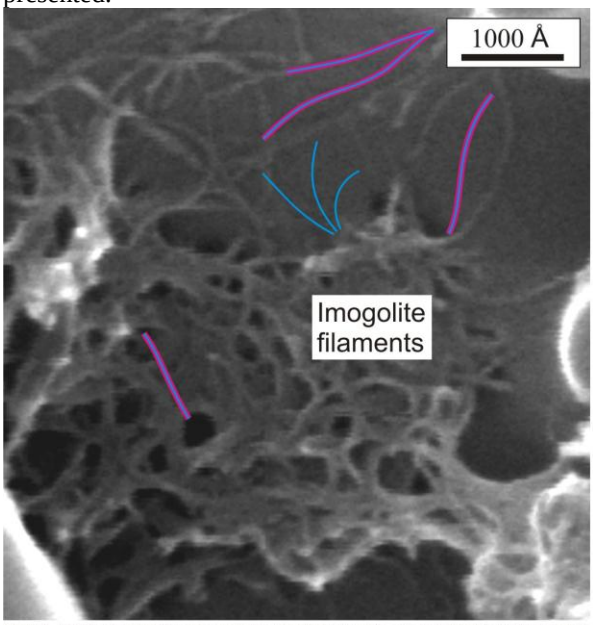


**Fig. 3.** High resolution secondary electron image of authigenic nanoscopic filaments of imogolite found within alpha-recoil track etch-tunnels in basaltic glass. Actual size of 20 Å wide hypothetical imogolite filaments shown in blue, with the 20 Å iridium coating shown in pink. Note similarity in size of hypothetical iridium coated imogolite with the natural nanofilaments. Sample DSDP-418A-75-3[120-123].

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**Diagenetic Origin of Moretane Anomalies in the Late Permian and Early Triassic** K. L. French[1], N.J. Tosca[2], C. Cao[3], R.E. Summons[4] [1]Joint Program in Chemical Oceanography, Massachusetts Institute of Technology and Woods Hole Oceanographic Institution, Cambridge, Ma 02139, United States. [2]Department of Earth Sciences, University of St Andrews, St Andrews, KY16 9AL, Scotland UK. [3]State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing, Jiansu, 210008, China. [4] Department of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Ma 02139, United States

The spatial distribution of clays has played a key role in the evaluation of potential landing sites for the Mars Science Laboratory (MSL) and the eventual selection of Gale Crater. The reasons for considering clays in this decision are two-fold. First, the presence and types of clays as indicated by spectroscopic data provide strong indicators of past environmental conditions. Second, the exceptional preservation of organic matter by clays is highly relevant for accomplishing the scientific objectives of the MSL. However, clays also mediate the stereochemistries of organic molecules. Stereochemistry, in particular enantiomeric excess and diastereoisomeric preference, are measurable and valuable molecular biosignatures by which we might recognize other carbon-based life forms [1]. Yet in the terrestrial system, biological stereochemistries are altered over time as organic matter undergoes degradation and these diagenetic processes are mediated by organic-mineral interactions. The exact mechanisms by which certain minerals affect biomarker stereochemistries are not well constrained, but the Permian-Triassic Boundary (PTB) section at Meishan in South China provides an opportunity to test the complex relationship between lithology and the stereochemistry of bound molecules.

The 17β(H),21α(H)-moretane/17α(H),21 β (H) hopane ratio is conventionally used as a thermal maturity indicator, but like other geochemical parameters, organic source input and depositional environment can modulate the measured moretane/hopane ratios of lipid extracts [2]. We present the moretane/hopane record of $C_{30\text{-}34}$ homohopanes and $C_{31\text{-}33}$ methylhomohopanes from a drill core spanning the initiation and recovery intervals of the Late Permian Extinction (LPE; 252.25 Ma) as recorded in the Meishan section [3]. Three intervals of anomalous moretane/hopane ratios are observed. Carbonate measurements and clay analysis implicate lithology as a primary factor for generating the moretane/hopane anomalies at Meishan. It seems likely that certain clay types, particularly chlorite, illite, and illite/smectite mixed layer clay, preferentially bind and protect triterpanes having the unstable 17β(H),21α(H) stereochemistry. The $C_{35}$ Homohopane Index, a redox indicator, shows an inverse correlation moretane/hopane ratios implicating a detrital origin for the moretane anomalies. These results have important implications for the interpretation of stereochemical biosignatures in the context of life detection on early earth and in extraterrestrial materials.

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**"LIFE IN CI CHONDRITES": NOT LIFE, NOT EXTRATERRESTRIAL, NOT EVEN INTERESTING.**
Marc Fries, Planetary Science Institute, Tucson AZ. *fries@psi.edu*.

**Summary:** A recent article in an online journal [1] claims to find fossils of native, extraterrestrial microbes in CI meteorites permineralized by sulfates. An alternative explanation is presented here, namely that the filamentous forms observed in [1] are well-documented, common CI sulfate minerals in filamentous form. The primary rationale presented in [1] is the author's perception that filamentous sulfates within the meteorite share morphological similarity with terrestrial microbes. Furthermore, the composition of these filaments is described as being unlike any known mineral. However, Raman spectroscopy shows that CI sulfate filaments are common sulfate minerals in accordance with existing literature [2-6]. These sulfates can be observed to grow in a matter of minutes or hours on freshly-exposed CI meteorite surfaces as ambient humidity drives the hydration of meteoritic sulfides [2]. While contamination by terrestrial microbes can provide a plausible explanation for filamentous morphologies when identified as biological by an expert, in this case the most plausible explanation is that the "microbes" are in fact sulfate filaments previously documented in literature.

**Discussion:** The claim that extraterrestrial microbe fossils are found in CI meteorites was made in Hoover (2011) [1]. The author describes filamentous structures on "internal surfaces" of Ivuna and Orgueil CI1 samples using field-emission scanning electron microscopy (FESEM). Hoover describes biomorphological features of these filaments and proceeds to claim that they are fossilized based on the fact that no nitrogen is detectable by energy diffraction spectroscopy (EDS) measurement. The lack of nitrogen is also used to state that the forms are not modern contaminating microbes. Data presented in Hoover (2011) consistently show that EDS measurements of the filaments include Mg, S, O, Si, ±C ([1], Figures 1, 2, 3). The interpretation offered is that the filaments are fossils formed by sulfate mineralization with remnant carbonaceous species. No interpretation is offered for the presence of silicon other than the claim that these filaments are "…not consistent with known species of minerals." The author concludes that these filamentous morphologies are the fossilized remnants of microbes native to the CI parent body.

The following features of CI filamentous morphologices are relevant to this discussion:

*Sulfate filaments in CIs:* Sulfate minerals have been noted previously in the CI meteorites as early as 1961 [3]. While debate has flourished as to their origin (terrestrial or parent body, or both) [2-6], the presence of sulfate veins and filaments is well supported by available literature. Notably, Gounelle and Zolensky (2001) [2] note that sulfates are found as alteration minerals in "all CI chondrites", both as veins and surficial decoration. This paper shows sulfate filaments growing from the surface of a prepared CI thin section (Figure 1 in [2]), indicating that these filaments appear on very short time scales even with careful sample handling. They conclude that sulfates to include filaments form via terrestrial alteration in contact with humidity, "…filling the many open spaces offered to them by the very porous rock." Others have also noted that sulfate filaments can grow rapidly on freshly-fractured surfac-

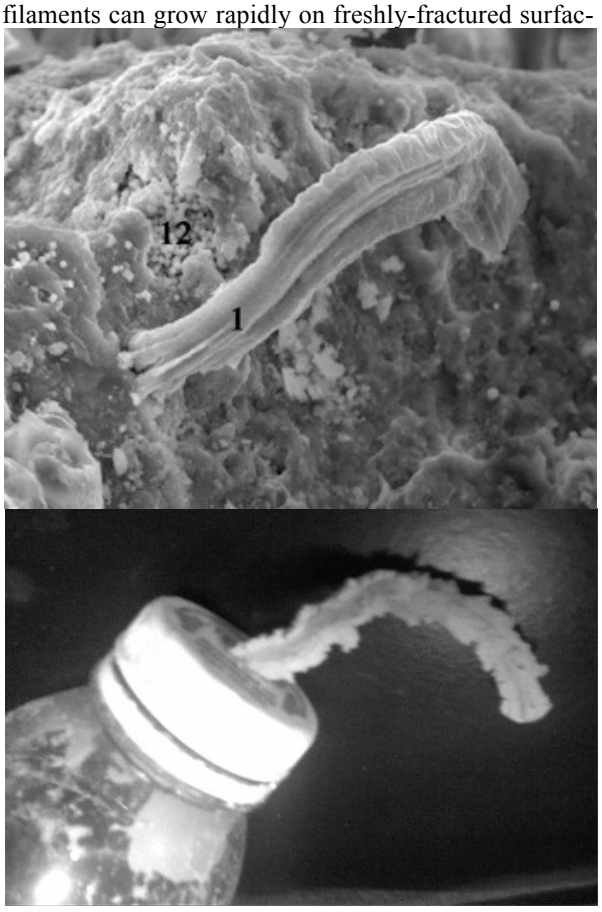


Figure 1: Extruded morphology of "microbes". Top: Image adapted from Figure 2a of Hoover (2011) showing purported extraterrestrial microbe. Bottom: Image of clay extruded from a homemade extruder showing similar morphology. "Microbes" are extruded from the porous CI matrix as sulfides alter to sulfates under the influence of ambient humidity. Image credit: Lauren Vork, www.ehow.com

es of CI meteorites [M. Fries, unpublished data, and C. Smith, pers. comm.).

To summarize, sulfates form readily in CI meteorites via oxidation/hydration of CI sulfides by ambient moisture. The filamentous morphology of some CI sulfates occurs by extrusion of the growing sulfate through CI matrix porosity (Figure 1). Therefore, their morphology can be altered by varying the meteorite's exposure to atmospheric humidity as the filaments grow. Simply moving a sample in and out of a desiccator cabinet, for example, could produce the lobate, "pinched" or other morphologies identified as biogenetic in Hoover (2011). Filaments presented in [1], however, are predominently invariant in cross-section, consistent with extruded material produced under relatively invariant conditions ([1], Fig. 2a, 3a).

*Mineralogy of Sulfate Filaments:* Previous work has identified CI sulfates as a mixture to include hexahydrite ($MgSO_4 \bullet 6H_2O$), epsomite ($MgSO_4 \bullet 7H_2O$) and gypsum ($CaSO_4 \bullet 2H_20$) (see [2] Table 2 for a list). A Tonk CI1 filament observed as identical in morphology to those presented in Hoover (2011) was recently identified as gypsum via Raman spectroscopy (Figure 2). The salient point is that sulfate filaments in CI chondrites are both easily identified and readily represented in available literature. This conclusion is readily supported by data in Hoover (2011) itself, which includes EDS data of multiple filaments (see above). The compositions listed are readily explained as Mg sulfates (Mg, S, O) with the EDS interaction volume extending into the CI matrix behind the filaments to include a contribution of carbon and silicon. This interpretation also supports the absence of nitrogen without the need to invoke an alien biosphere somehow habituating the cold, irradiated, atmosphere-free CI parent body.

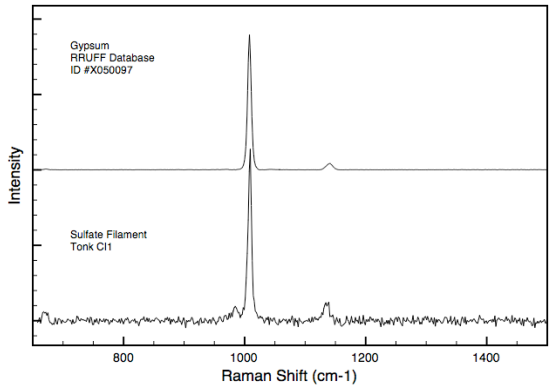


Figure 2: Raman spectrum of a Tonk CI1 filament (Bottom) identical in appearance to those in Hoover (2011) showing a mineralogical match to gypsum (Top).

**Conclusions:** The Hoover (2011) paper arrives at the conclusion that filaments in CI meteorites are biological in origin. Filaments in CI meteorites are better explained by an abiological origin.

*Morphology:* Small-scale variations in filament morphology that are identified as biological in origin are more readily explained as variations in the extrusion rate of sulfate. Changes in the humidty experienced by the rock could produce the various forms noted. This is a robust, simple explanation that does not require invoking the existence of an entire biosphere on a hostile parent body.

*Composition:* Hoover (2011) claims that the mineralogy of the filaments represents an unknown mineral species. This argument is presumably based on the EDS composition of Mg, S, O, Si, ±C. Raman spectroscopic data (and available literature) show that CI filaments are a mixture of common sulfates. EDS data presented by Hoover (2011) are sufficient to identify the filaments as Mg-sulfates with the addition of Si and C from the CI matrix in the EDS interaction volume behind the filaments.

The claim is made that the filaments are fossilized microbes based on the lack of nitrogen [1]. This argument requires that the filaments are microbial, and then because the "microbes" contain no nitrogen they must be ancient and have lost their nitrogen through alteration of their organic matter to a devolatilized kerogen. It is a far simpler explanation that CI sulfate filaments are simply aqueous alteration products of CI sulfides as has been reported repeatedly in the available literature. With this explanation in place, no explanation for the lack of nitrogen is logically required.

*Overall:* The hypothesis that filaments in CI meteorites are biogenic in origin is not supported by the data presented in Hoover (2011). Far and away the simplest and most scientifically plausible explanation is that filamentous sulfates in CI meteorites are aqueous alteration products, and that minor variations in their morphology are due to slight changes in ambient humidity during growth. Their composition is straightforward, as is their formation mechanism.

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**THE MICROBIAL CONTAMINATION STATE OF AS-FOUND ANTARCTIC METEORITES.** [1]Fries M., [2]Harvey R., [3]Jull A.J.T., [4]Steele A., [5]Wainright N., and the ANSMET 07-08 Team. [1]Planetary Science Institute, Tucson AZ, [2]Case Western Reserve University, Cleveland, OH, [3]University of Arizona, Tucson, AZ, [4]Carnegie Institute for Science, Washington, DC, [5]Charles River Laboratories, Wilmington, MA. *fries@psi.edu*

**Summary:** Microbial contamination was measured in Antarctic meteorites to test the hypothesis that these meteorites are sterile or nearly so in their as-found condition. Meteorites were collected aseptically and then sampled and analyzed in a sterilized glove box. Extracts from these measurements were kept frozen until the measurements could be repeated in a laboratory. Laboratory measurements proved to be consistent with field measurements, establishing the validity of the measurements obtained in the field. Two measurement techniques were used: limulus amebocyte lysate (LAL) assay featuring single-cell sensitivity to gram-negative microbes, and adenosine triphosphate (ATP) luminometry which is sensitive to the metabolism of all cells. The body of measurements indicates that *Antarctic meteorites are sterile in their as-found condition*, with the caveat that this suite of measurements is less sensitive to the presence of gram-positive microbes such as fungi. The same measurements performed on meteorites collected with the standard ANSMET protocol indicate that microbial contamination is introduced during collection. Realistically, however, microbial contamination should be viewed as an inevitability to be minimized. Eliminating it completely would strain available resources for little real gain. Furthermore, aseptic collection of Antarctic meteorites is a very time-consuming process that would have a severe negative effect on the number of Antarctic meteorites available for research. Replacement of the existing protocol is not warranted, but rather any scientific study where microbial contamination is a factor should include quantitative analysis of the contamination state of that specific sample.

Figure 1: Aseptic meteorite collection. The collector is wearing a full-body clean-room garment, boot covers, sterile gloves, and a face-mask. The meteorite is handled with sterilized tongs and stored in a plasma-cleaned Al container with witness plates. The container is sealed in a furnace-sterilized Al bag wrapped in Al foil. Collection is performed downwind from the meteorite.

**Methods:** Six meteorites were collected aseptically (Figure 1) during collection sessions specified for this activity. All snowmobiles approached from downwind and were turned off when a candidate meteorite was sighted. Of the six meteorites, three were ordinary chondrites suitable for use in this study. Sufficient reagents were available to supplement the study by analyzing three meteorites collected via the typical ANSMET protocol. Three ordinary chondrites were selected at random from the box of meteorites collected that season, with the permission and oversight of the Meteorite Working Group chairman. These meteorites were sampled and analyzed in a single session. The glove box was sterilized using a proven field sterilization protocol [1] and measurements were performed in a warm tent with dry Antarctic air pumped into the glove box through a filter/desiccator apparatus (Figure 2). 5g of material was separated from each

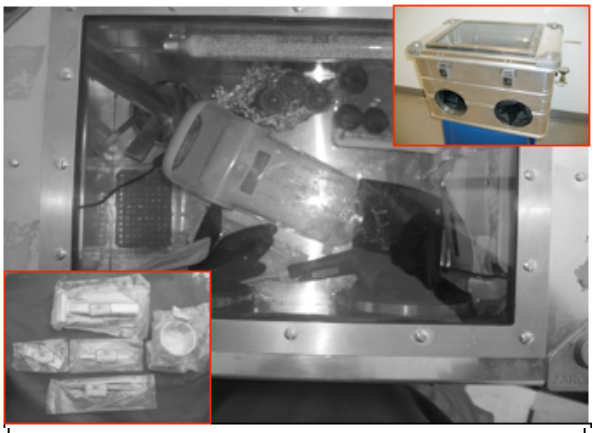

Figure 2: Sterilized metal glove box used for measurements. Large image: Top-down view showing samples, air handling filter/desiccator (top) and LAL instrument (center). Inset upper right: Overview of glove box. Inset lower left: Sterilized, sealed meteorite sampling tools provided by the Astromaterials Curation Laboratory, NASA Johnson Space Center.

meteorite and crushed, 5 mL of pyrogen-free water was added to each, the mixture was vortexed for 10 minutes and allowed to settle, and supernatant fluid was pipetted off for LAL and ATP measurements. No two sample containers were opened at the same time, and the glove box was cleaned between samples. A round of measurements were also performed on witness plates. After the measurements were completed, the collection of meteorite/water aliquots were frozen and remained so until re-analyzed in the microbiology laboratory at the Geophysical Laboratory of the Carnegie Institute of Science. All LAL and ATP measure-

ments were repeated to test the validity of the field measurements.

**Results and Discussion:** ATP measurements for both field and laboratory measurements (Figures 3 and 4, respectively) show no detectable microbial metabolism is occurring in any sample. This does not indicate that the samples are sterile, but rather that any microbes present are quiescent.

LAL results consistently produced zero values for microbial abundance in the meteorites collected by aseptic procedures (Figures 3 and 4). Field measurements of the meteorites collected using the ANSMET protocol, however, produced non-zero values for all three meteorites tested (Figure 3). Repeat measurements in the laboratory show that two of those three show zero values and the meteorite that produced a maximum-range response in the field (MIL 07114) retains a high value (Figure 4). The diminished response is most probably the result of some sample degradation in transport, which highlights the need for field measurements for this type of study.

Results of this study show that meteorites found on the Antarctic ice are sterile to within the limits of this study.

The evidence of microbial contamination in meteorites collected via the ANSMET protocol does not immediately require that the protocol be amended. The aseptic collection protocol required bulky equipment and supplies and *took roughly ten times as long as collection using the ANSMET protocol*. Applying aseptic collection procedures would effectively cut the number of meteorites collected roughly ten-fold, and would not prevent meteorites from accruing contamination once they leave Antarctica. In balance, the current protocols are in good balance with the need for relatively large numbers of new and/or unusual meteorites for meteorite research.

**References:**

[1] Eigenbrode J. *et al*, *Astrobiology* **9** (2009) 455-465.

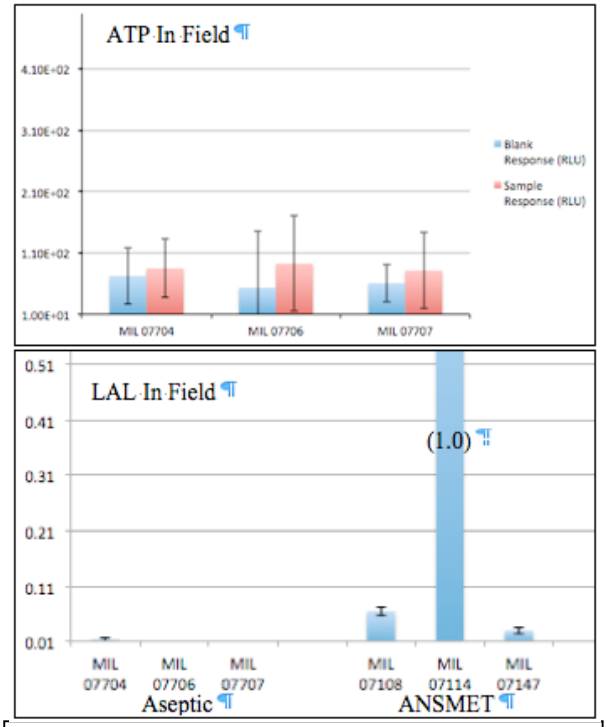


Figure 3: LAL and ATP measurements in the field. ATP measurements (TOP) show that aseptically collected meteorites feature instrument values statistically indistinguishable from background values. ATP was unavailable for meteorites collected with the ANSMET protocol. LAL measurements (BOTTOM) show zero values for aseptically collected meteorites but non-zero values for all three meteorites collected via the ANSMET protocol.

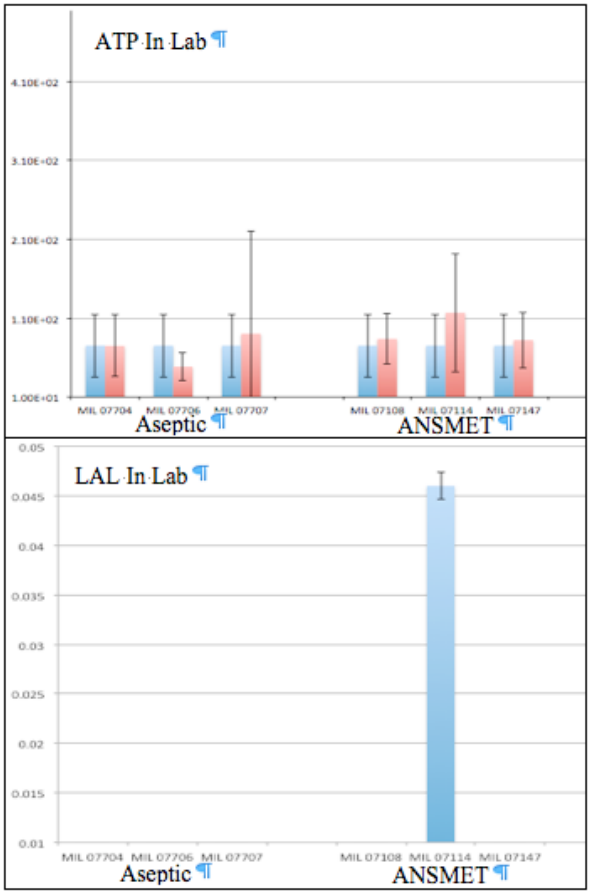


Figure 4: ATP and LAL measurements repeated in the laboratory. ATP measurements (TOP) are consistently statistically indistinguishable from background values. LAL results (BOTTOM) imply some degradation of the samples such that only one of the three ANSMET protocol samples retains a positive value. This is a reasonable result. The combination shows that any contaminating microbes present are not actively metabolizing.

**STRATEGIES FOR DISTINGUISHING ABIOTIC CHEMISTRY FROM MARTIAN BIOCHEMISTRY IN SAMPLES RETURNED FROM MARS.** D. P. Glavin, A. S. Burton, M. P. Callahan, J. E. Elsila, J. C. Stern, and J. P. Dworkin, NASA Goddard Space Flight Center, Greenbelt, MD 20771, daniel.p.glavin@nasa.gov.

**Introduction:** A key goal in the search for evidence of extinct or extant life on Mars will be the identification of chemical biosignatures including complex organic molecules common to all life on Earth. These include amino acids, the monomer building blocks of proteins and enzymes, and nucleobases, which serve as the structural basis of information storage in DNA and RNA. However, many of these organic compounds can also be formed abiotically as demonstrated by their prevalence in carbonaceous meteorites [1]. Therefore, an important challenge in the search for evidence of life on Mars will be distinguishing between abiotic chemistry of either meteoritic or martian origin from any chemical biosignatures from an extinct or extant martian biota. Although current robotic missions to Mars, including the 2011 Mars Science Laboratory (MSL) and the planned 2018 ExoMars rovers, will have the analytical capability needed to identify these key classes of organic molecules if present [2,3], return of a diverse suite of martian samples to Earth would allow for much more intensive laboratory studies using a broad array of extraction protocols and state-of-the-art analytical techniques for bulk and spatially resolved characterization, molecular detection, and isotopic and enantiomeric compositions that may be required for unambiguous confirmation of martian life.

Here we will describe current state-of-the-art laboratory analytical techniques that have been used to characterize the abundance and distribution of amino acids and nucleobases in meteorites, Apollo samples, and comet- exposed materials returned by the Stardust mission with an emphasis on their molecular characteristics that can be used to distinguish abiotic chemistry from biochemistry as we know it. The study of organic compounds in carbonaceous meteorites is highly relevant to Mars sample return analysis, since exogenous organic matter should have accumulated in the martian regolith over the last several billion years and the analytical techniques previously developed for the study of extraterrestrial materials can be applied to martian samples.

**Analytical Techniques:** In order to measure the soluble organic composition (including amino acids and nucleobases) found in terrestrial and extraterrestrial samples, solvent extraction of the powdered sample followed by purification and chemical derivatization is typically required depending on the target compound and analytical technique used. Details of the extraction procedures used for amino acids and nucleobases in meteorites are published elsewhere [4,5]. In brief, for amino acid analyses, hot water extracts were derivatized with *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC) and analyzed by liquid chromatography time-of-flight mass spectrometry (LC-ToF-MS); for nucleobases, formic acid extracts were analyzed by liquid chromatography with high resolution ($m/\Delta m \geq 60{,}000$), accurate mass (< 5 ppm) Orbitrap detection.

**Amino Acids and Chirality:** The amino acid compositions of carbonaceous chondrites have been characterized using a variety of techniques and some of these meteorites contain a rich structural diversity that is not seen in terrestrial amino acids.

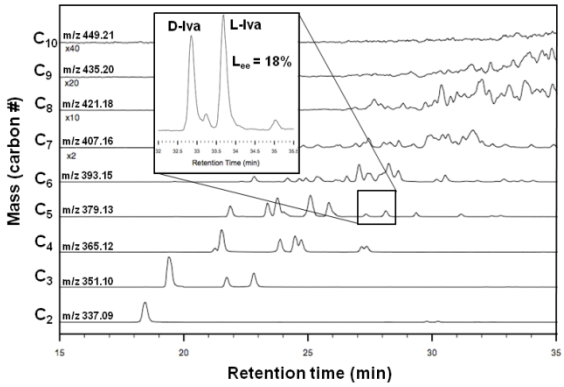


**Figure 1.** LC-ToF-MS chromatogram of OPA/NAC amino acid derivatives in the Murchison meteorite showing the mass traces corresponding to $C_2$ to $C_{10}$ amino acids. A large L-enantiomeric acid ($L_{ee}$) excess of 18% was measured for the $C_5$ amino acid isovaline, a terrestrial rare amino acid [4].

For example, over 80 different amino acids have been identified in the CM meteorites Murchison and Murray using gas chromatography mass spectrometry, and they comprise a mixture of two- to eight-carbon cyclic and acyclic monoamino alkanoic and alkandioic acids of nearly complete structural diversity, many of which are completely nonexistent in the terrestrial biosphere [1]. Our recent analyses of the Murchison meteorite using a much more sensitive LC-ToF-MS instrument provide evidence for a much larger diversity of amino acids with masses of up to ten carbons [6], indicating that hundreds of individual amino acids are present (Fig. 1). Many of the amino acids in carbonaceous meteorites are thought to have formed by Strecker synthesis during aqueous alteration on the parent body, although other mechanisms for the formation of amino acids in some more thermally altered meteorites have been proposed [7]. The complex amino acid distribution in Murchison is distinct from present day life where proteins are built from a much more limited set of amino acids. Although it is unknown whether or not a past

martian biota even existed, characterizing and comparing the complete distribution of amino acids in a returned sample to what is commonly produced in abiotic chemistry would provide important constraints on their origin(s).

In addition, many amino acids are structurally chiral. Life on Earth is dominated by L-amino acids, while all known abiotic syntheses of amino acids result in racemic mixtures of amino acids (i.e. L = D) in the absence of a chiral driving force. Therefore, chirality can be an important tool to help discriminate between amino acids of abiotic or biotic origins. However, even chirality measurements of amino acids found in a martian sample would need to be interpreted with caution, since many amino acids common to life on Earth will racemize on geologically short timescales changing their original enantiomeric ratios. In addition, large L-enantiomeric excesses of isovaline found in Murchison and other aqueously altered meteorites [6] demonstrate that abiotic mechanisms for producing enantiomeric biases exist. Ideally, detection of a large excess of D-amino acids in a martian sample would provide compelling evidence for an origin of life independent from Earth.

**Nucleobases:** Until recently, the origin of nucleobases identified in some carbonaceous meteorites [8] had not been firmly established. One of the issues was that all of the purines (adenine, guanine, hypoxanthine, and xanthine) and one pyrimidine (uracil) detected are biologically common and could be explained as the result of terrestrial contamination.

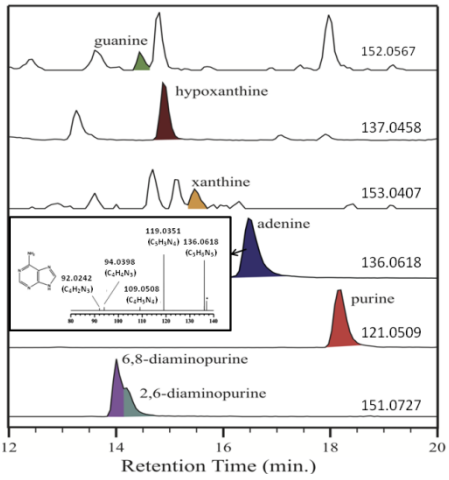


**Figure 2.** Single ion accurate mass chromatograms of a formic acid extract of a meteorite analog (HCN polymer) obtained using liquid chromatography Orbitrap mass spectrometry. Purines were identified by their monoisotopic parent masses (± 5 ppm) and multiple fragment masses (inset) and chromatographic retention time [5].

Using liquid chromatography Orbitrap mass spectrometry, a diverse suite of purines were unambiguously identified by accurate mass in Murchison [5]. In addition to the purines previously identified in Murchison [8,9], three unusual and terrestrially rare nucleobase analogs were also found: purine, 6,8-diaminopurine, and 2,6-diaminopurine. An identical suite of nucleobases and nucleobase analogs were produced in reactions of ammonium cyanide (Fig. 2), which provides a plausible mechanism for their synthesis and strongly supports an extraterrestrial origin for these compounds in meteorites. The discovery of new nucleobases in meteorites expands the prebiotic molecular inventory available for constructing the first genetic molecules on Earth and potentially on Mars.

Due to the extremely low abundances (< 250 ppb) of nucleobases in meteorites [5], determination of their C, N, and H isotopic ratios has been extremely challenging. Martins *et al.* reported non-terrestrial carbon isotope values for xanthine and uracil in Murchison [9], however this measurement alone required ~15 grams of bulk sample. Compound-specific stable isotopic analysis of organic compounds in samples returned from Mars will be extremely important in establishing their origin, especially if the molecules identified are similar to those found in life on Earth.

**Conclusions:** Ultimately, the search for indisputable chemical evidence of life on Mars may require measurements that even go beyond current laboratory analytical capabilities, including molecular *spatial resolution* of amino acids, nucleobases, carboxylic acids, and other organic molecules important to life. Currently, the spatial distribution of these key organic compounds found in meteorites is not understood. In the future, instruments with much lower detection limits combined with new sample handling/extraction technologies will enable detection of these organic compounds in individual micron sized grains. In addition, other non-destructive analytical techniques need to be developed to enable spatially resolved measurements of these organic compounds. Even with improvements in instrument and sample handling technologies, identifying molecular biosignatures from an extinct martian biota in a returned sample that was significantly altered over time in the harsh radiation and oxidizing martian surface environment will be challenging.

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**BIOCONTAINMENT CHALLENGES FOR HANDLING AND LIFE DETECTION OF EXTRATERRESTRIAL SAMPLES.** M. A. Grimaldo L., M. Eng.[1], R. L. Beets, FAIA[2], and J. S. Sandlin, AIA[3]; [1]Director of Institutional Biocontainment Resources, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0609, magrimal@utmb.edu, [2]Perkins+Will, 1001 McKinney St., Suite 1300, Houston, TX 77002, Raymond.Beets@perkinswill.com, [3]Perkins+Will, 1001 McKinney St., Suite 1300, Houston, TX 77002, Scott.Sandlin@perkinswill.com.

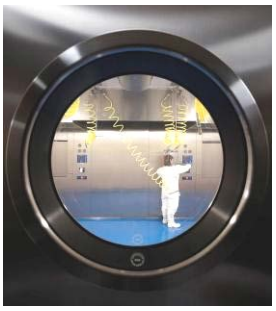
**Introduction:** The intent of our presentation will be to initiate a multidiscipline discussion on how samples would be collected, transported, preserved and analyzed where the environment and the scientist can be protected and the life detection work could be carried out in a safe manner that protects the researcher and the general public. We propose to present and discuss issues of sample integrity and containment, and lessons learned from our experience in the design, construction, commissioning and operation of high-containment biological research facilities. We will focus on our experience with Biosafety Level 4 (BSL-4) laboratories as well as the challenges that maximum containment has on sample handling, specialized equipment operation and maintenance, sample storage, environmental conditions, disinfection methodologies, air filtration, effluent decontamination, validation of systems and preparation for unknowns.

**History:** "The prospect of eventually returning samples from diverse bodies throughout the solar system underscores the need for a specialized sample return facility dedicated to the study and detection of life in extreme environments. …In anticipation of the variety of proposed sample return missions…, it will be important to be prepared with a suitably stringent containment and quarantine facility…"[1]

To date, the most relevant extraterrestrial samples returned to Earth have been the samples collected by the Apollo and Luna missions, and more recently, the Genesis and Stardust missions. These have shown to be devoid of life and to contain only extremely low amounts of organic materials. In contrast, it is expected that future missions will involve return of samples from bodies that cannot with the same degree of confidence be assumed not to contain life. Studies by the Space Studies Board of the National Research Council[1,2] have recommended that such samples be handled under strict biological containment, even though the possibility that they contain life is very small. In addition, the science to be done on returned samples requires that they be handled under extremely clean conditions to maximize their scientific value, especially to avoid "false positives" for the detection of life, i.e., mistaking terrestrial contaminants for evidence of life on these extraterrestrial bodies.

A planetary sample collection will be an invaluable, international scientific resource. It is well-established that planetary samples must be stored and scientifically manipulated in a manner so that their state of preservation will not be compromised or degraded.

**Summary:** The design, construction, and operation of a Sample Receiving Facility will require the coordination and work of multiple teams of experts, spanning a decade or more. It is important for various layers of scientific and technical oversight to be in place early in the planning process to ensure continuity throughout the lengthy and complex Mars sample return mission planning process.[3] To meet the recommendations of the 2009 report to be "fully operational at least 2 years prior to the return of samples to Earth", we hope this dialogue will contribute to the process of planning and constructing a safe and secure sample receiving facility, built according to applicable containment level standards, with the ability to maintain strict cleanliness conditions, equipment integrity, and operational protocols necessary to optimize the unique opportunities presented by a Mars sample return.



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**µ-XANES at the S-K edge and hard/soft XRF analyses of ancient biosignatures in Early Archaean cherts from Barberton, South Africa.** A. Hubert[1,2], A. Simionovici[2], L. Lemelle[3], F. Westall[1], B. Cavalazzi[4], J.N.-Rouzaud[5], C. Ramboz[6], [1]Centre de Biophysique moléculaire-CNRS-OSUC, Orléans, France (frances.westall@cnrs-orleans fr), [2]ISTE-Grenoble, France, [3]ENS-Lyon, France, [4]Univ. Johannesburg, South Africa, [5]ISTO-CNRS-Orléans, France, [6]ENS-Géologie, paris, France..

**Introduction:** One of the main objectives of a sample return mission from Mars is the *in situ* search for biosignatures [1]. Reliable identification of traces of life in very ancient rocks requires a wide range of evidence that includes: (1) information on the nature of the degraded organic molecules that constituted the former life forms, (2) evidence of metabolic activity, and (3) eventually morphological traces of the fossilised life forms [2-4]. Methods developed for the study of ancient traces of terrestrial life can be adapted to the search for extraterrestrial life. We here describe nanoscale *in situ* analysis of molecular and elemental signatures associated with a previously well-characterised, 3.3 Ga-old microbial mat from the Barberton Greenstone Belt [4,5]. This is a photosynthetic microbial mat that was first calcified and then hydrothermally-silicified as it was living. Although it is unlikely that photosynthetic microorganisms developped on Mars 3], calcification related to sulphur (not sulphate) reducing bacteria activity could have occurred in the vicinity of high biomass production, for instance around hydrothermal vents.

**Methods:** For this study we cut thin vertical FIB slices 3 µm thick though the mat. We used X-ray Fluorescence spectrometry (XRF) and X-ray Absorption Near-Edge Structure spectroscopy (XANES) of submicrometric resolution at the European Synchrotron Radiation Facility (ESRF) of Grenoble [4]. We focused on sulphur (S) and calcium (Ca) phases as representative of sulphur reducing bacteria activity and carbonate precipitation.

**Results:** The first set of XRF analyses was made at low energy: 2.5 keV to be more sensitive to the sulphur phase. Coupled with XANES analyses at the S-K edge, they allowed us to identify and localise two different phases of sulphur: an inorganic phase (sulphate, Fig. 1a) and an organic phase (thiophene, Fig. 1b).

A second set of analyses was made at higher energy: 17,4 keV in order to detect heavier elements, such as the Ca. This showed a spatial overlap between the distribution of organic S and Ca (Fig. 2) indicating a potentially common phase. Indeed, high resolution TEM study of other FIB slices of the microbial mat [4] documented the precipitation of the carbonate phase (aragonite) onto the organic matrix of the silicified mat.

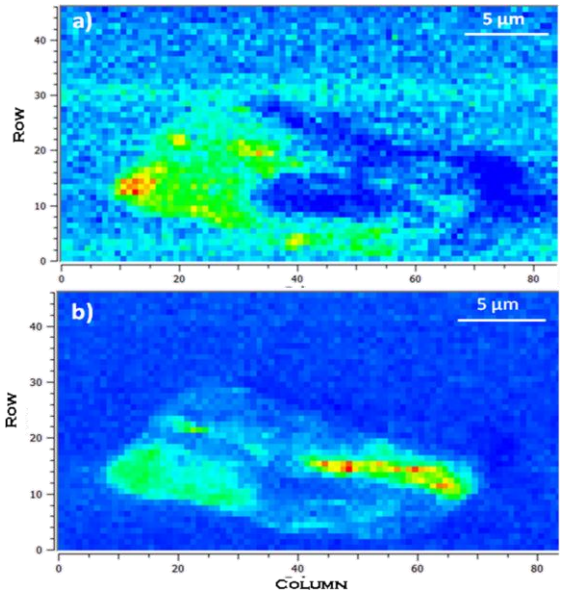


Figure 1. Spatial distribution of the S phases: a) sulfates map at E = 2,482 keV; b) organic S map at E = 2,474 keV.

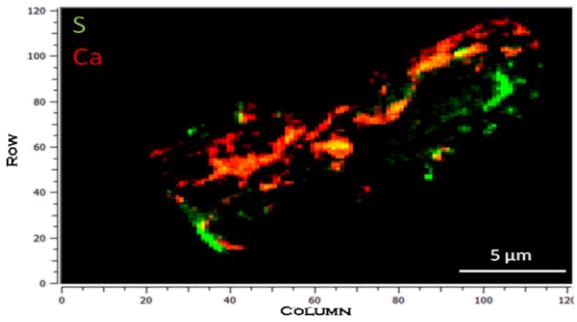


Figure 2. Compared distributions of S (green) and Ca (red). E = 17,4 keV. The overlapping distributions are orange.

**Discussion and conclusions:** Sulphur, an important bioelement, was detected in both an organic and inorganic phase on a nanometer-scale in this 3.3 Ga-old microbial mat. Thiophene is an organic molecule linked to the reduction of organic matter by biogenic processes [6] but can also be found in abiogenic meteoritic organic matter. Its direct association with the Ca phase aragonite, however, suggests a calcification process guided by sulphur reducing bacteria with the pre-

cipitation of crystallites of carbonate on the S-rich organic matrix.

Our study underlines the necessity of using nanoscale *in situ* analyses in the study of ancient biosignatures, as well a pluridisciplinary approach since it was only with HR-TEM that we were able to accurately identify the carbonate phase and to determine the critical association with the degraded organic matter of the microbial mat.

As noted in the introduction, such calcification could occur in hydrothermal settings where anaerobic biomass is (relatively) high. Tentative evidence of hydrothermal activity has been detected on Mars [7] and hydrothermal materials are amongst those proposed for sample return and the search for martian life.

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**Survival of Methanogens on Different Martian Regolith Analogs: Implications for Life Detection in Returned Martian Samples.** T. A. Kral[1,2] and T. S. Altheide[2], [1]Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR, [2]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR. tkral@uark.edu.

**Introduction:** We have been studying methanogens as models for life on Mars for the past 18 years (1, 2, 3, 4, 5, 6, 7, 8, 9). Methanogens are microorganisms in the domain Archaea that can metabolize $H_2$ as an energy source, $CO_2$ as a carbon source, and produce methane. They are one possible explanation for the methane found in the Martian atmosphere (10, 11, 12). If an organism is to exist in the hostile Martian environment, it must be able to deal with a number of relatively extreme factors. Three of those factors are limited availability of liquid water, low pressure, and minimal nutrients. Here we report on research designed to determine if certain species of methanogens can survive desiccation at Mars surface pressure of 6 mbar, in different Martian regolith analogs, for both 90 and 120 days.

**Methods:** The low-pressure desiccation experiments were performed in the Pegasus Chamber, located at the Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville. The methanogens tested, *Methanosarcina barkeri, Methanobacterium formicicum, Methanothermobacter wolfeii and Methanococcus maripaludis,* were grown in their respective growth media in anaerobic culture tubes. Following five days of growth, cultures were centrifuged followed by suspension of the cell pellets in 700 uL of sterile buffer containing sodium sulfide (to remove residual molecular oxygen). In a Coy anaerobic chamber, 10 uL of each cell suspension were added to anaerobic culture tubes containing sterile Martian regolith analogs (montmorillonite, nontronite, basalt, jarosite or JSC Mars-1 soil simulant) or glass beads (control). The tubes were removed from the anaerobic chamber and placed into the Pegasus chamber. The chamber was sealed and evacuated down to 6 mbar, resulting in desiccation of all of the cultures. Following 90 days and 120 days (separate experiments), the tubes were removed from the chamber, rehydrated with ideal growth media, and placed under ideal growth temperatures for the respective methanogens. Additionally, in the 90-day experiment, some of the rehydrated cells from the glass beads were transferred to regolith analogs, and some of the rehydrated cells from regolith analogs were transferred to fresh medium without regolith analogs. At regular time intervals, headspace gas samples were removed and analyzed for methane using gas chromatography.

**Results and Discussion:** All three organisms that were placed on glass beads demonstrated substantial methane production with time (50 percent or greater of the headspace gas) following both 90 (Table 1) and 120 (Table 2) days of desiccation. *M. barkeri* survived on multiple Martian analogs (JSC Mars-1, montmorillonite and basalt) following the two desiccation periods. Also, some *M. wolfeii* and *M. barkeri* cells transferred to and from regolith analogs demonstrated survival. No organism survived on the nontronite. Based on the three factors tested here, the results would seem to indicate that some methanogens may be able to survive and possibly thrive on Mars. If methanogens inhabit the regolith of Mars, and if some are able to survive desiccation at low pressure as seen here, then returned samples of Martian regolith may yield viable cells.

| | Glass | Substrate* | Glass→Substrate | Substrate→Medium |
|---|---|---|---|---|
| *M. wolfeii* | +++ | - | + (SS & Basalt) | + (Clay & Basalt) |
| *M. barkeri* | +++ | ++ (SS & Clay) | + (Clay & Basalt) | ++ (SS & Clay) |
| *M. formicicum* | ++ | - | - | - |
| *M. maripaludis* | - | - | - | - |

*JSC Mars-1 (SS)
Clay (Montmorillonite)
Basalt

Table 1. Methane production by *Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum* and *Methanococcus maripaludis* following desiccation at 6 mbar for 90 days.

| | Glass Beads | Ch. Basalt | Gr. Basalt | Ch. Jarosite | Gr. Jarosite |
|---|---|---|---|---|---|
| *M. wolfeii* | +++ | - | - | - | - |
| *M. barkeri* | +++ | ++ | ++ | ++ | - |
| *M. formicicum* | ++ | - | - | ++ | - |
| *M. maripaludis* | - | - | - | - | - |

Ch. = Chunk
Gr. = Ground

Table 2. Methane production by *Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum* and *Methanococcus maripaludis* following desiccation at 6 mbar for 120 days.

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Evolution of Sub-micron-Size Cyanobacteria in Polar Ice over 50 Million Generations

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Using fluorescence spectrometry to map autofluorescence of chlorophyll (Chl) in polar ice cores, followed by flow cytometry (FCM) and epifluorescence microscopy (EFM) of melts of that ice, we found that sub-micron size picocyanobacteria (*Prochlorococcus* and *Synechococcus*) were responsible for most if not all of the Chl in the cores. In ice melts from 2 Arctic and 6 Antarctic sites passed through a 1.2 µm filter to remove large cells, we calibrated the FCM patterns of Chl (red) *vs* phycoerythrin (PE, orange) with FCM patterns of cultures of *Pro* and *Syn*. From the FCM measurements we concluded that *Pro* and *Syn* are the dominant sub-micron ecotypes in all polar ice samples, sometimes with 2 to 3 strains of *Syn* in the same sample. FCM plots of Chl *vs* side scattering (SSC) showed that *Pro* are usually 0.5 to 0.8 µm and *Syn* are ~0.6 to ~1.5 µm, consistent with the sizes of those genera in the oceans. We measured concentrations from ~2 to 2 x $10^4$ *Pro* cells/$cm^3$ of ice (median 70 *Pro*/$cm^3$) and a median value 2.6 for the ratio *Pro/Syn*. Chl and PE autofluorescence intensities showed no apparent decrease per cell with time during 150,000 years of storage in glacial ice. From our fluorimetric scans of Chl concentrations in ice cores we found values up to ~30% higher at depths corresponding to local summers than to local winters. Figure 1 shows examples of our fluorimetry compared with SeaWiFS satellite Chl measurements for the North Atlantic and the Antarctic Oceans. Taking into account this annual modulation, together with ocean temperatures and winds, we inferred that both *Pro* and *Syn* cells are wind-transported from mid-latitude, temperate waters onto polar ice.

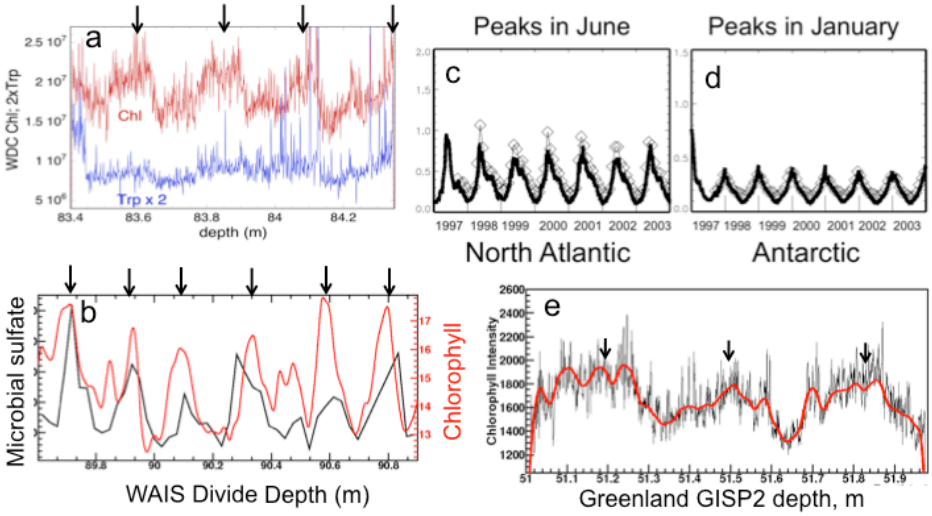


Figure 1. Summer maxima of Chl autofluorescence in ice cores scanned by the Berkeley Fluorescence Spectrometer. (a) Chl and Trp over 4 years in 1-meter length of West Antarctic Ice Sheet (WAIS) Divide ice; (b) Chl and microbial sulfate maxima in WAIS Divide ice are in phase; (c) and (d) are data from SeaWiFS satellite showing that Chl in North Atlantic Ocean surface are maxima in northern summer, and in Antarctic Ocean are maxima in southern summer; (e) Chl peaks for 3 summers in Greenland GISP ice core.

To us, the annual modulation of Chl autofluorescence in terrestrial ice cores is reminiscent of the exciting finding by others that methane concentration in the Martian atmosphere increases during Martian summer and wanes in winter. It has still not been established whether the methane has a biogenic or abiogenic origin. By being able to obtain samples of terrestrial ice cores, we have concluded that the increase in Chl is due to the annual

warming of terrestrial oceans inhabited by *Pro* and *Syn* and to the transport of some of those cells from ocean surfaces onto the growing Arctic and Antarctic ice sheet. Similarly, by being able to return samples from Mars, it may be possible to pin down the origin of the periodic methane releases and possibly to discover indigenous life forms on Mars.

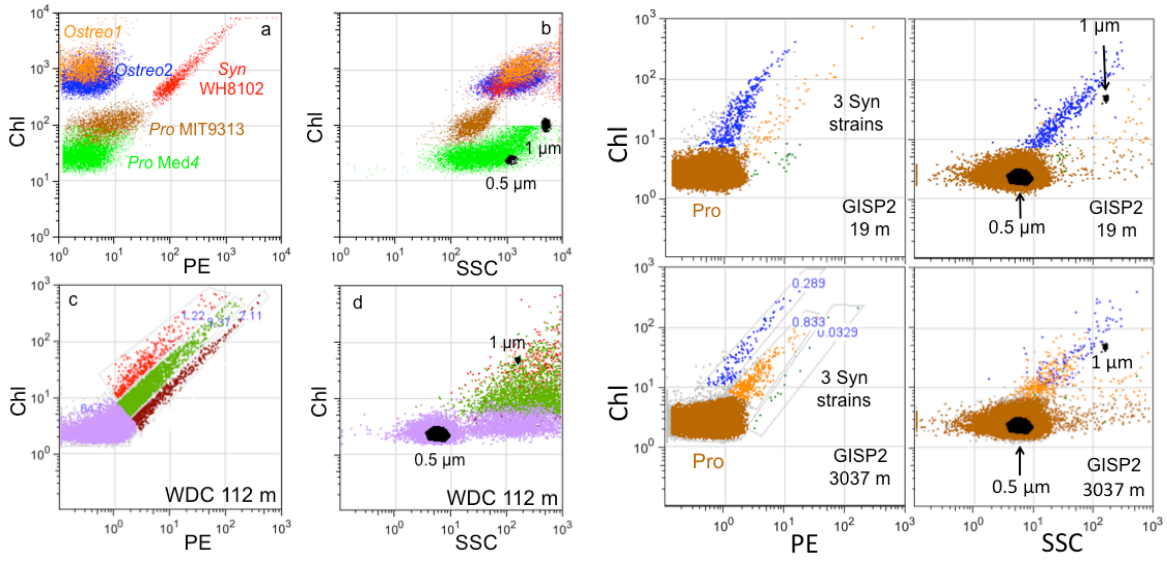


Figure 2. Examples of flow cytograms in pairs (Chl *vs* PE; Chl *vs* SSC). (a,b) show patterns for cultures of *Ostreococcus* strains 1 and 2, *Pro* strains MIT9313 and Med4, and *Syn* strain WH8102, using 0.5 and 1.0 µm fluorescent beads for size calibration; (c,d) WAIS Divide ice from 112 m depth; and GISP2 ice from depths of 19 m and 3037 m.

Finally, comparing the flow plots on the right sides of Fig. 2, we see that the concentrations of *Pro* and *Syn* cells are very similar for ice of ages 60 yrs (i.e., 19 m) and 150,000 yrs (i.e., 3037 m). There appear to be 3 strains of *Syn* at both depths. With a growth rate of 1 to 2 per day for *Pro* and *Syn* in mid-latitude oceans, and no growth for cells in glacial ice, we would like to do genomics on *Pro* and *Syn* at, say, 10 depths in Arctic ice and 10 depths in Antarctic ice, to study evolutionary changes over 50 million generations in the ocean. We would use the cells in ice at -30°C as a frozen proxy of the same ecotypes living in the ocean up to 150,000 years ago. It will be an intriguing problem in microbial ecology to tease out evolution of the same strains at different depths.

We thank former graduate students Nathan Bramall and Robert Rohde and post-doctoral researcher Delia Tosi for construction, operation, and analysis of data from the Berkeley Fluorescence Spectrometer; Steve Ruzin and Denise Schichnes for training students in EFM and providing live/dead stains; Hector Nolla for training students in FCM and for doing cell sorting and operating the Cytopeia Influx Sorter; undergraduate students Tong Liu, Yevgenya Rudenko, Elaine Lee, Lisa Chen, and Joyce Lee for laboratory assistance; Elaine Lee and Lisa Chen for EFM and FCM analyses; and Kathryn Johnson (*Pro*, *Syn*, *Micromonas pusilla*), Kate Mackey (five strains of *Syn*), Alexandra Worden (*Ostreo*), and Steve Giovannoni (*Pelagibacter ubique)* for providing cultures. We acknowledge partial support from NSF Grant ANT-0738568.

**FORGING A DRAFT TEST PROTOCOL FOR MARS SAMPLE HAZARD TESTING ON EARTH.** J. D. Rummel, East Carolina University, Greenville, NC 27858, <rummelj@ecu.edu>.

**Introduction:** In anticipation of a Mars sample return (MSR) mission taking place during the first decade of the 21st Century, and with the recommendation of the US National Research Council's Space Studies Board [1], NASA undertook the development of a protocol for the handling and testing of martian materials returned to Earth. Previous groups and committees had studied selected aspects of sample return requirements, and a workshop held at NASA Ames in 1997 [2] was of particular importance in coalescing those studies. Specific detailed protocols for handling and testing were needed to understand both the feasibility of testing allowing for the release of a martian sample from containment as "safe," and the nature of a facility that would allow for the effective application of that protocol. To further refine the requirements for sample hazard testing and to develop the criteria for subsequent release of sample materials from containment, the NASA Planetary Protection Officer convened a series of workshops beginning in 2000 with the overall objective of developing a comprehensive draft protocol to assess returned martian sample materials for biological hazards while safeguarding those samples from possible Earth contamination. This process culminated in the release in October 2002 of NASA's "A Draft Test Protocol for Detecting Possible Biohazards in Martian Samples Returned to Earth"[3]. This presentation will review the process that led to that document, the nature of the discussions that led to the final document, and perceptions of the document's ongoing utility to international preparations for a future MSR mission..

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**LIFE DETECTION USING BIOPATTERNING.** K. E. Schubert[1], E. Gomez[1], J. Curnutt[1], and P. J. Boston[2], M. Spilde[3], H. Qiao[1], [1]School of Computer Science and Engineering, California State University, San Bernardino, San Bernardino, CA 92407; keith@r2labs.org [2]Earth & Environ. Sci. Dept., New Mexico Institute of Mining & Technology, Socorro, NM 87801, [3]Depatment of Earth and Planety Science, University of New Mexico.

Figure 1:Patterning in Salt Creek, Death Valley.

Figure 2: Patterned soil crust east of Baker, CA, courtesy Geoffrey Payton.

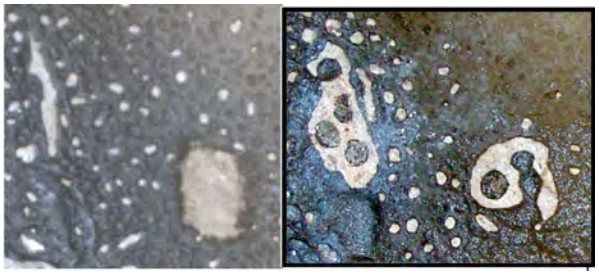

Figure 3: Regrowth experiment, courtesy Loise Hose, showing pattern variation from Apr/1999 to Sep/2003.

**Bio-Patterning as a Marker of Life:** As resources become scarce, lifeforms adapt by growing in patterns that help them optimize their access to resources. It does not matter if the resource is nutrients, water, or light, the patterns formed are a reflection of the scarcity of the resource, not the type of resource that is laking. From algae in Salt Creek, Death Valley, CA (Fig. 1), to soil crusts near Baker, CA (Fig. 2), to geometrically elaborate biomats on cave walls (Fig. 3) life in extreme environments frequently does not exhibit a solid mat of growth, but rather forms exotic patterns ranging from linear mazes to circles, and harder to characterize amorphous patterns [1]. Similar patterns are seen in desert grasses around the world, and even in microbial mats in caves, (Fig. 3). Fortunately, such patterns often lithify and persist past the active growing phase of a community especially in microbial communities within caves.

**Search for Life:** One of the biggest problems in the search for life on other planets, such as Mars, is where to start looking. In any environment where life once existed but now is rare or gone, that life would most likely have gone through a period of limited resources. Even in catastrophic events, not all life dies instantly, but rather many individuals and communities struggle on and leave a final trace of their efforts to survive. In isolated environments, like cave systems, organisms would be less effected and thus live longer. Further, their bio-signature would be better protected than in a more open environment like the planet surface. Caves thus recommend themselves as an ideal environment to look, but the principles outlined here also apply to a wider environment. On any planet which had extant life at one point in its history, but now may have only scarce or fossil life, life probably has exhibited patterned growth. This implies that a reasonable search for life on other planets should involve looking for patterned growth in places where some type of life might still be holding on, or looking for fossilized patterns to find where life once lived [2].

If this was all that patterned growth was useful for, then it would be of limited utility, however the patterns themselves also tell us about the structure and resource conditions of the environment when the life was actively growing. This is important for understanding the overall conditions on the planet (or ancient time period on an earlier Earth), and thus can be used to make predictions of where else life might be found. On our own world, these patterns can serve as a barometer of past climate, indicators of prior environmental conditions when such biopatterns were formed, and can teach us about the fundamental energetics and ecology of microbial communities.

**Cellular Automata Models:** To describe, model, and extract system understanding, we are utilizing cellular automata. A cellular automaton is a model, consisting of a grid of cells, that can take on discrete values. Specifically, we have used a system where cells

could either be alive (1) or dead (0). The sum of all the neighbors within three cells of a cell is used as an index to a rule book, that specifies if that central cell will grow, die, or stay the same during the next time period. This summation and rule lookup process is done for every cell, at each time interval. The sum can vary from 0 to 49, which quantifies how dense the life is in that area. Zero is empty and 49 is full. Most rules were left as *stay-the-same*, including both 0 and 49, which must be left at *stay-the-same* for stability.

We have noted that in many natural systems there tends to be low density (sparse) regions, where only a few isolated organisms or small colonies exist, and high density areas (dense), where a solid mat is only broken up by isolated holes. These are generated by potential *wells*: in a section of the rules, the less dense area experiences net growth and the more dense area experiences net death. The growth rules in the less dense area thus increases the density towards the death rules in the more dense area, generating a relatively stable pattern with a density around the middle of each *well*.

Most patterns are composed of a series of wells in different regions, and their effect can be directly explained. For example:

1) A well in a low density region tends to cause the pattern to spread across the field and is thus necessary for life to perpetuate itself. This is almost certain to be the case in any system, as without it, it is unlikely that a community would have enough members to survive.

2) A well in a high density region tends to prevent growth from making a complete coverage of the field, and thus keeps the community from using up scarce resources too fast. The "holes" in the pattern move, providing for a natural equivalent of crop rotation.

3) A well in the middle densities allows a mechanism for smooth transition between the high and low density wells, thus allowing organisms to adapt to a changing environment and depleting resources without changing the fundamental strategy (the rules).

**Pattern Simulation:** To simulate the soil crusts found in the Mojave (Fig. 2) or cave wall patterns (Fig. 3), we needed a set of rules that could explain the wide sparse areas with widely varying growth patch sizes and very dense regions, which had more consistent hole sizes. This was handled by making the low density well have a relatively wide region of *stay-the-same* rules between growth and death. For instance, in Figure 4, the well rule was *grow* (1- 3) and *die* (8-12), leaving the wide region of 4-7 as *stay-the-same*. The high density well was located at *grow* (34- 38) and *die* (41-44), and thus had half the size of the bottom of the well and a slightly wider grow region to keep the edges sharp. The middle or transition well, was located at *grow* (25-27) and *die* (28-29). Notice it does not have any bottom to speak of, which encourages the communities to "bounce out" of the well, acting as a transition to the next well above or below in density. The lower end of the transition well was chosen to account for two of the low density spots getting close enough and merging, but not to allow one community to grow on its own. The result is a good approximation of the real soil crusts found in Figure 2.

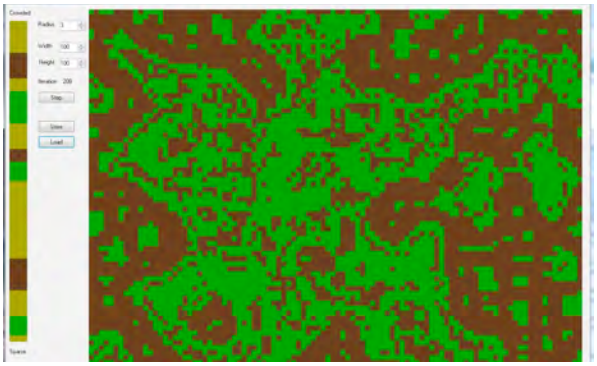

Figure 4: Simulation of cellular automaton with rule-set consisting of three "wells". Green means live, brown is die, and yellow doesn't change. Rules are on the left.

**Conclusions:** The cellular automata approach to biopatterning is of potentially great utility for the identification of possible biomarkers in extraterrestrial settings and in Earth's ancient rock record. The fundamental ecological and energetic drivers that make such biopatterning successful is likely to be a broadly distributed property of life, irrespective of particulars of chemistry, genetics, or other biomolecular structural details. On Earth we see such patterns extensively on cave walls of all different lithologies and in a variety of other environmental conditions (e.g. temperature, moisture, geochemistry, etc.), in cryptogamic soils in arid regions, and even in higher plant patterning. Thus, the search for preserved evidence of such patterns can be developed into a robust metric of prior (or even current) life processes. Such patterns lend themselves to analysis by computerized robotic missions as well as being highly distinctive to the human eye. Because of the extraordinarily good preservation conditions within caves, such patterns are likely to be better preserved in subsurface cavities than they are on a geologically active and weathered planetary surface.

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**ABIOTIC VERSUS BIOTIC PATHOGENS: REPLICATIVE GROWTH IN HOST TISSUES KEY TO DISCRIMINATING BETWEEN BIOTOXIC INJURY AND ACTIVE PATHOGENESIS.** Andrew C. Schuerger[1], Douglas W. Ming[2], and D. C. Golden[2]. [1]University of Florida, Bldg. M6-1025, Space Life Sciences Lab, Kennedy Space Center, FL 32899; email: schuerg@ufl.edu; [2]Astromaterials Research and Exploration Science Office, Mail Code JE23, NASA JSC, Houston, TX 77058; emails douglas.w ming@nasa.gov and d.c.golden1@nasa.gov, respectively,

**Introduction.** Life can be defined as a self-sustaining chemical system capable of undergoing Darwinian evolution; a self-bounded, self-replicating, and self-perpetuating entity [1]. This definition should hold for terrestrial as well as extraterrestrial life-forms. Although, it is reasonable to expect that a Mars life-form would be more adaptable to Mars-like conditions than to Earth-like environments, it remains possible that negative ecological or host interactions might occur if Mars microbiota were to be inadvertently released into the terrestrial environment.

A biogenic infectious agent can be defined as a self-sustaining chemical system capable of undergoing Darwinian evolution and derives its sustenance from a living cell or from the by-products of cell death. Disease can be defined as the detrimental alteration of one or more ordered metabolic processes in a living host caused by the continued irritation of a primary causal factor or factors; disease is a dynamic process [2]. In contrast, an injury is due to an instantaneous event; injury is not a dynamic process [2]. A *causal agent of disease* is defined as a pathogen, and can be either abiotic or biotic in nature.

Diseases incited by biotic pathogens are the exceptions, not the norms, in terrestrial host-microbe interactions. Disease induction in a plant host can be conceptually characterized using the Disease Triangle (Fig. 1) in which disease occurs only when all host, pathogen, and environmental factors that contribute to the development of disease are within conducive ranges for a necessary minimum period of time. For example, plant infection and disease caused by the wheat leaf rust fungus, *Puccinia recondita*, occur only if virulent spores adhere to genetically susceptible host tissues for at least 4-6 hours under favorable conditions of temperature and moisture [3]. As long as one or more conditions required for disease initiation are not available, disease symptoms will not develop.

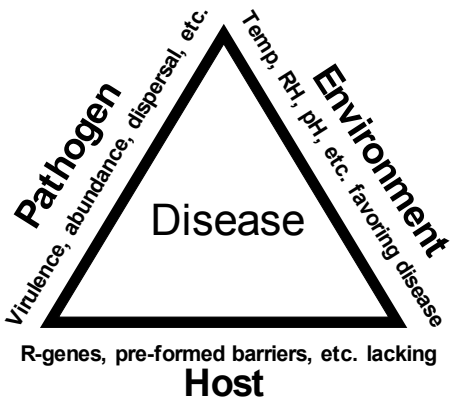


Figure 1. Disease Triangle.

**Life Detection in Mars Samples.** In order to release returned Mars samples to the general scientific community, several criteria must be met that convince the community at large of the safety of the materials. The following sequence is proposed as a preliminary experimental structure to determine the biosafety of returned Mars samples.

First, terrestrial life is based on carbon, and it is likely that an extraterrestrial pathogen also must be based on carbon if it is to gain sustenance from terrestrial life-forms. If true, then analyses of martian regolith and rocks for organics will be the first line of defense in assaying returned samples for the presence of potential pathogens. Second, a series of replicative assays should be conducted in culture (i.e., outside host tissue) to determine if putative Mars microbiota are present in the samples and capable of growth and cellular replication under a diversity of environmental conditions spanning the range from the martian surface to terrestrial ecosystems. And third, bioassays should be conducted with plant, animal, invertebrate, and microbial systems to confirm the absence of harmful biological entities in the samples. If all three of these tests are negative, the samples are likely to be safe to release to the community.

However, to date, there are no established protocols for assaying returned samples to demonstrate their biosafety to terrestrial life-forms or ecosystems. The primary objective of this project was to investigate the effects of aqueous extracts of Mars analog soils on the plant host *Capsicum annuum* (pepper; a traditional host indicator crop for viral, bacterial, and fungal pathogens) in order to begin the development of protocols that might discriminate between abiotic (not a safety issue) versus biotic pathogens.

**Materials and Methods.** Six Mars analog soils were generated from terrestrial minerals, crushed and sieved to pass 500 µm stainless steel sieves, and stored at 24 C until used. The six Mars soils were created to represent: (1) a benign basalt-only soil, (2) high-salt soil, (3) acidic soil, (4) alkaline soil, (5) perchlorate soil, and (6) an aeolian dust simulant. Aqueous extracts of each simulant were created by vigorously shaking 50 g of soil in 100 ml of sterile deionized water (SDIW; 18 Ω) for 2 h in a baffled 250 ml flask. The extracts were filtered through Whatman #4 paper, 0.45 µm, and 0.22 µm filters. The pH and electrical conductivity (EC) of analog soils are given in Table 1.

**Table 1.** pH and EC for six Mars analog soils.

| Analog soils | pH | EC |
|---|---|---|
| Basalt (control) | 8.1 | 68.2 µS $cm^{-1}$ |
| High salt | 2.9 | 18.3 mS $cm^{-1}$ |
| Acidic | 2.7 | 38.8 mS $cm^{-1}$ |
| Alkaline | 10.2 | 11.6 mS $cm^{-1}$ |
| Phoenix | 6.7 | 5.5 mS $cm^{-1}$ |
| Aeolian dust | 6.7 | 6.9 mS $cm^{-1}$ |

The aqueous extracts (0.5 ml/injection) of the Mars simulants were then injected into leaves of 28-d old pepper plants (*C. annuum*, cv. Hungarian Wax) using 20G needles (Fig. 2). The procedure is used as a standard method of injecting fluids with presumptive pathogens into leaf tissues in order to screen for microbial virulence. For viral pathogens, pepper leaves can exhibit local lesions (e.g., Fig. 3, tomato mosaic virus [ToMV] on tobacco), leaf chlorosis (yellowing), veinal collapse, systemic development of symptoms, and eventual necrosis of both leaf and canopy tissues. For bacterial pathogens, a water soaked lesion around the point of injection is typically observed within a few days. Leaf and plant wilt is possible with both classes of biological pathogens.

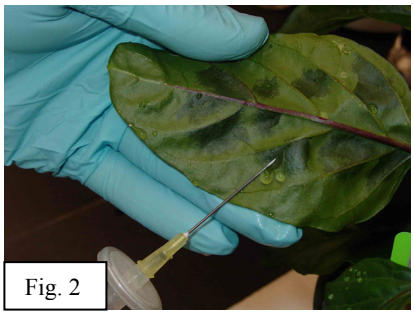

Fig. 2

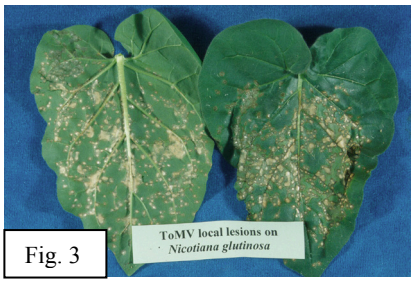


Fig. 3

**Results.** Injection of SDIW (black marks in Figs 4 and 5) produced no discernable symptoms in pepper leaves. The SDIW was simply reabsorbed by the leaf tissues, and leaves appeared normal within 2-4 h (Fig. 4). In contrast, injections of the high-salt simulant extracts induced interveinal chlorosis on the treated leaf, followed by tissue necrosis at the points of fluid injection. Acidic soil extracts induced rapid necrosis of the fluid saturated injection sites (within 2 h) and total-leaf necrosis within 48 h. However, if the symptomatic tissues for both salt and acid simulants were ground in SDIW in sterile mortars and pestles, and then injected into healthy and symptom-free pepper leaves, no additional symptoms were observed.

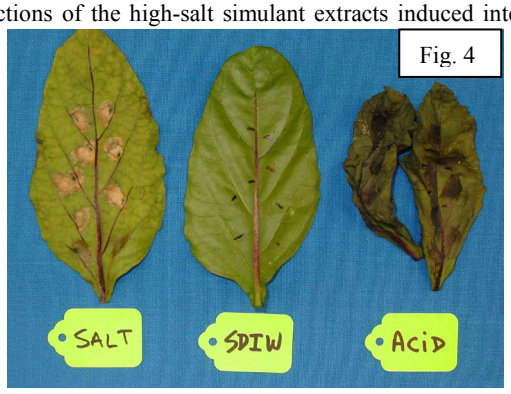


Fig. 4

In a separate series of tests, high salt and high acid simulant extracts were titrated to pH 7, and then 0.5 ml injected into fresh symptom-free pepper leaves. Results for the acid and salt aqueous extracts (e.g., Fig. 5; high-salt simulant) indicated that necrosis was observed for the low-pH soil extracts, but was absent for the pH 7 titrated simulant extracts. Results suggest that the primary edaphic factor that was responsible for leaf necrosis was low pH in both soil extracts (Table 1).

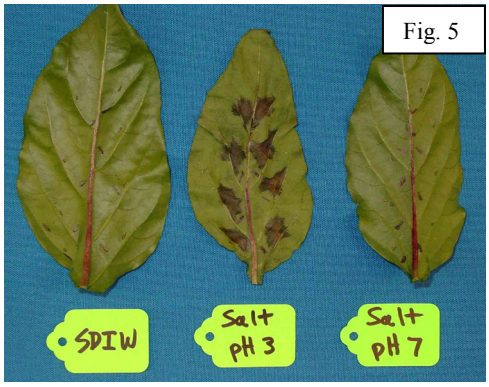


Fig. 5

Results from leaf injections of other soil extracts indicated minor biotoxic responses in pepper leaves for neutral pH extracts from the perchlorate and aeolian dust simulants. The high pH alkaline soil extracts induced symptoms similar to the aqueous extracts from the salt and acidic simulants, but to a lesser degree than salt or acid soil extracts. In all cases, if symptomatic leaf tissues from the 1st set of injections were ground in SDIW using sterile mortars and pestles, and then injected into healthy pepper leaves, tissues failed to develop any of the symptoms described above.

**Conclusions.** Bioassays are proposed here as an essential part of assessing the biosafety of returned samples from Mars. Due to the extremes of pH, EC, or other edaphic factors that are likely to be present in some Mars samples, biotoxic injuries of challenged tissues are likely. As abiotic factors are diluted through subsequent challenges, symptoms of biotoxic injury are likely to disappear quickly through time due to dilution. In contrast, a biological pathogen capable of growth and replication through multiple generations is likely to repeatedly induce disease symptoms over multiple challenges. For example, the local lesions induced by ToMV in tobacco (Fig. 3) would continue to induce new local lesions indefinitely over multiple generations. Thus, replicative growth of a presumptive microbial pathogen from Mars might induce symptoms that can be differentiated from one-time injury effects by edaphic factors in samples. If symptoms persist over time and through multiple sequential challenges, a biological entity must be assumed to be present, even if replicative evidence in culture (i.e., free of host tissue) is lacking.

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**Microbial Life in the Atacama Desert: Using a Multidiciplinary Approach to Examine the Habitability Potential and Microbial Diversity in a Mars Analog Environment.** T. B. Shirey[1] and J. B. Olson[1] The University of Alabama, Department of Biological Sciences, Tuscaloosa, AL 35487.

**Introduction:** When setting out to explore the potential for life on Mars, it is the biosphere of Earth that is used as a rubric for discovery. Research into habitable conditions for life in the solar system includes studies of analogous environments on Earth with comparable conditions suitable for harboring life. Fortunately, there are select environments on Earth that mimic many of the conditions found on Mars, and although no environment on Earth is perfectly comparable to Mars, we can utilize unique natural analog environments on Earth as alternatives to direct exploration.

One such Mars analog environment is the Atacama Desert, Chile, the environment in which this study was conducted. The Atacama is a narrow stretch of desert in northern Chile spanning over 1000 km, from 18ºS to 27ºS. Over time, the Atacama has been transformed by associated geologic formations and atmospheric conditions into one of the most unique and inhospitable landscapes on the planet. As a result, this desert has served as a Martian analog for several NASA studies [1, 2].

Apart from the apparent geological similarities between the Atacama Desert and Mars, a common attribute shared by both is lack of water availability. The Atacama is an arid to hyper-arid desert and considered the driest region on Earth [3]. The lack of available water in the Atacama creates an intensely inhospitable climate for life to persist. It has been postulated that perhaps in areas of extreme hyperaridity in the Atacama, the dry limit of microbial habitability has been reached [4]. Although the Atacama suffers from an extreme lack of water availability throughout its landscape, a precipitation gradient exists along a latitudinal transect. This gradient produces degrees of "dryness" within the desert that can serve as a backdrop to examine its effects on the microbial communities that exist there.

The primary focus of this study was to examine the distribution and diversity of microbial communities in the Atacama Desert along a latitudinal transect subjected to a measured precipitation gradient. One particular emphasis of this study is to utilize a multifaceted approach, applying both direct and indirect methods of microbiological community characterization, to examine the microbial communities within the desert. Benefits of using a multi-pronged approach become clear when one considers the limitations encountered from any individual method of examination. Moreover, this particular approach uses both traditional microbiological cultivation techniques and contemporary molecular methodologies, which, when combined and examined in context, should generate a clearer picture of the overall microbial community in the Atacama.

**Sampling:** Soil samples were aseptically collected from six different sites (AT-01, -02, -03, -04, -05, -08) in the Atacama Desert along a 600 km latitudinal transect. Between the six sampling sites, 71 samples of Atacama soils were collected. Soils were collected from 27ºS near the city of La Serena, continuing north to 18ºS. Within this transect is the hyperarid Yungay region, which is known as the driest region of the Atacama. The southernmost sites (AT-01 and AT-02) are characterized as "wetter" environments, while the northern sites (AT-04, 05, and 08) are within the range of extreme hyperaridity. Site AT-03 represents a transitional site between hyperarid and extreme hyperarid.

**Methods:** Two types of methodologies (direct and indirect) were utilized for this study. Direct methods of microbial characterization included using phospholipid fatty acid (PLFA) analysis for both quantifying microbial biomass and examining community structure, and direct cell counts using DAPI epiflourescence microscopy.

Indirect methods of analysis examined both DNA extracted directly from Atacama soils, and from enrichment cultures cultivated in media with varying nutrient concentrations. A variety of media (both solid and liquid) were specifically developed for this study with compositions ranging from nutrient rich to highly oligotrophic. DNA extracted from both the soil and the enrichment cultures was quantified and used for PCR analysis. Amplified products of the 16S rRNA gene tagged with a fluorescence label were subsequently used for terminal restriction fragment length polymorphism (T-RFLP) analysis to examine the bacterial community composition along the sampling transect.

**Results:** Although experiments are continuing, data collected thus far indicate an association exists between bacterial habitability and latitude within the interior of the Atacama Desert. This latitudinal correspondence is likely due to the precipitation gradient that exists along the south-north transect.

Extracted DNA per gram of soil was highest at the southernmost latitude (14.1 ng/µl) and lowest in the northern latitudes (2.9 ng/µl). Likewise, based on gel electrophoresis banding intensities, the southern-

most sites yielded the most amplifiable DNA, with amplification decreasing with decreasing latitude. Moreover, with the exception of site AT-01, the liquid cultures produced extractable DNA that was comparable to that taken directly from the soil.

Extracted DNA concentrations corresponded to colony forming unit (CFU) counts of bacteria cultured from the Atacama soils. A range of CFU/g soil was seen along the sampling transect ($3.0 \times 10^4$ to 0 CFU/g). Like DNA concentrations, total CFU/g was highest in the south and decreased with decreasing latitude. Again, this is attributed to the precipitation gradient along the sampling transect.

Bacterial cultivations on various types of solid media showed a degree of morphological variation that was not entirely unexpected. The solid media used for this study was developed with a range of nutrient and mineral concentrations, that should each select for bacteria with differing growth requirements.

**Conclusions:** The Atacama Desert is an ideal environment to examine the microbial limits of habitability. This type of study can be beneficial to future search for life missions, as it examines both habitability potential and method efficacy within a natural planetary analog. Although conditions in the Atacama Desert are extreme and inhospitable to life, bacteria, although perhaps existing in a state of dormancy, do exist along many sampled regions of the desert. Within the context of all data collected thus far, CFU counts, PCR amplifiable products and DNA concentrations appear to correlate.

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## IN-SITU SOIL MICROBIAL DETECTION USING NATIVE FLUORESCENCE

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For this research project we designed an instrument to detect bacteria via biomolecular fluorescence. We introduce the current understanding of astrobiology, our knowledge of life beyond Earth, and the commonality of Earth life as it pertains to the search for life on Mars. We proposed a novel technique for searching for direct evidence of life on the surface of Mars using fluorescence. We use the arid region of the Mojave Desert as an analog of Mars. Results indicate the fluorescence of the biotic component of desert soils is approximately as strong as the fluorescence of the mineral component. Fluorescence laboratory measurements using the portable instrument reveal microbial concentration in the Mojave Desert soil is $10^7$ bacteria per gram of soil. Soil microbial concentrations over a 50 meter$^2$ area in the Mojave Desert, determined in situ via fluorescence, show that the number varies from $10^4$ to $10^7$ cells per gram of soil. We then designed an instrument for detection of biomolecular fluorescence, and considered also fluorescence from polycyclic aromatic hydrocarbons and minerals on the Martian surface. The majority of the instrument is designed from Mars surface operation flight qualified components, drastically reducing development costs. The basic design adapts the ChemCam instrument package on-board *Mars Science Laboratory* rover *Curiosity* to detect organics via fluorescence. By placing frequency multipliers in front of the 1064 nm laser, wavelengths suitable for fluorescence excitation (266 nm, 355 nm, and 532 nm) will be achieved. The emission system is modified by the addition of band pass filters in front of the existing spectrometers to block out the excitation energy. Biomolecules and polycyclic aromatic hydrocarbons are highly fluorescent at wavelengths in the ultra violet (266 nm, 355 nm), but not as much in the visible 532 nm range. Preliminary results show minerals discovered, such as perchlorate, fluoresce highest when excited by 355 nm. Overall, we conclude the fluorescent instrument described is suitable to detect soil microbes, organics, biomolecules, and some minerals via fluorescence, offering a high scientific return for minimal cost with non-contact applications in extreme environments on Earth and on future missions to Mars.

**PROTEINS AS INFECTIOUS AGENTS AND IDENTIFICATION OF PRION-LIKE SELF-PROPAGATING PROTEINS IN EXTRATERRESTRIAL SAMPLES.** C. Soto[1] and R. Diaz-Espinoza[1], [1]Department of Neurology, University of Texas Medical School at Houston. 6431 Fannin Street, Houston, TX. E-mail, Claudio.Soto@uth.tmc.edu.

**Introduction:** The discovery that proteins can behave like micro-organisms to transmit disease is a significant milestone in biology. The unorthodox prion hypothesis was proposed decades ago to explain the surprising transmission mechanisms of a group of rare diseases known as transmissible spongiform encephalopathies, or prion diseases (1;2). The prion hypothesis states that the infectious agent in prion diseases is composed exclusively of a misfolded form of the prion protein, which replicates in infected individuals by transforming the normal version of the prion protein into more of the misfolded isoform. This hypothesis remained controversial for decades (3), but recent studies have settled all doubts by demonstrating that infectious material can be generated *in vitro,* in the absence of genetic material, by replication of the protein misfolding process (4;5). The infectious protein (called prion) exhibits the typical characteristics of bona-fide infectious agents (3), namely: exponential multiplication in an appropriate host, transmission between individuals by various routes including food-borne and blood-borne, titration by infectivity bioassays, resistance to biological clearance mechanisms, penetration of biological membrane barriers, "mutation" by structural changes forming diverse strains, and transmission controlled by species barriers. Despite that prions fulfill the Koch's postulates for infectious agents, it remains surprising that a single protein possesses the complexity and flexibility required to act like living micro-organisms that transmit disease.

Recent exciting research has led not only to the end of the skepticism that proteins can transmit disease but also to expanding the concept that infectious proteins might be at the root of some of the most prevalent human diseases. The transformation of a natively folded protein into a misfolded, toxic form that causes tissue damage and disease is not a mechanism exclusive to prion diseases. Misfolded protein aggregates are implicated in more than 20 human diseases, collectively called protein misfolding disorders (PMDs), including highly prevalent and insidious illnesses such as Alzheimer's disease, Parkinson's disease, and type 2 diabetes (6;7). Although the proteins implicated in each of these pathologies and the clinical manifestations of the diseases differ, the molecular mechanism of protein misfolding is strikingly similar.

The notion that protein misfolding and aggregation is associated with a small family of proteins has also dramatically changed in recent years. The pioneering work of Dobson and colleagues has demonstrated that many (if not all) proteins can form β-sheet intermolecular interactions to adopt an amyloid-like conformation under appropriate conditions (7). Strikingly, the structures formed and the underlying mechanism of misfolding and aggregation are similar to those for PMDs. These findings suggest that any protein has the potential to form misfolded aggregates that might replicate by the prion principle. Taken together, these results form the basis of a general biological principle whereby protein function and activity might be regulated by folding changes that can be rapidly propagated from protein to protein.

Few years ago we developed a technology termed Protein Misfolding Cyclic Amplification (PMCA) to efficiently reproduce prion replication in the test tube. The PMCA technology has been applied to convert large amounts of the normal prion protein into the abnormal form by incubating it with minute amounts of misfolded infectious prion protein. The system consists on cycles of accelerated prion replication to reach an exponential increase in the conversion. These findings mark the first time in which the folding and biochemical properties of a protein have been cyclically amplified in a manner conceptually analogous to the amplification of DNA by PCR. PMCA has contributed enormously to understand the underlying biology of prions, to identify other factors that may be implicated in prion protein conversion, and to discover novel drugs for prion diseases (8). In addition, PMCA has enormous potential in allowing current diagnostic tools to detect prion disease during the pre-symptomatic period and perhaps in living individuals, because it can multiply the number of prions facilitating their detection. Indeed, PMCA enables more than 3 billion fold increase on sensitivity for PrP detection and the possibility to detect as little as a single molecule of the misfolded protein (9). This level of sensitivity allowed us to detect for the first time prions in the blood and urine of sick as well as pre-symptomatic animals (10-12). These findings have had a major impact in various fields including the diagnosis of prion disease, blood banks safety, meat industry, environmental detection of prions, etc (8). More recently we have adapted the PMCA technology to amplify the processes of misfolding and aggregation of other proteins implicated in PMDs as

well as to identify new proteins with the ability to self-propagate their conformations.

Taken into account the facts that proteins can behave like infectious agents to transmit disease, that many disease-associated and non-disease associated proteins can self-propagate using the prion principle and that many (if not all) proteins can adopt a misfolded aggregated form it becomes highly relevant to search for new forms of self-propagating proteins. We believe that the PMCA technology provides a promising tool to identify novel infectious proteins in many samples, including extra-terrestrial materials.

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**Life Detection With Minimal Assumptions – Setting an Abiotic Background.** A. Steele[1] [1]Carnegie Institution of Washington, Geophysical Laboratory, 5251 Broad Branch Rd, Washington DC, 20015. asteele@ciw.edu

The simplest form of extraterrestrial life detection with minimal assumptions on the nature of the organism or a potential "alien biochemistry" to be detected, is to understand the possible abiotic organic chemical reactions given the context of the samples and look for perturbations to that signal. More precisely, life chooses only a few of the many known organic chemicals produced by abiotic processes. Therefore anomalous deviations from predicted abiological yields of organic chemicals under given conditions may be the easiest life detection protocol. The assumptions are minimal; life is carbon based and it chooses only a subset of possible abiotic chemicals available (Steele et al., 2006). An example would be the organic chemistry responsible for the inventory of organics in the Murchison meteorite and abiotic processes such as the Miller-Urey reaction and Fischer – Tropsch (FTT) synthesis. In the case of the Murchison meteorite, it appears that all possible isomers of a particular carbon number or compound are present but only a very limited subset of these molecules used by terrestrial biology (Schmidt-Koplin 2010). In the case of FTT as chain length increases, yield decreases and although analysis of the products this is subject to analytical problems, mainly volatile loss, the kinetics of this reaction are very well understood and predictable. Life on the other hand tends to use ~ C17 to C31 alkanes and produce an odd even preference that is not present in FTT products (Donnelly, 1989). A final and perhaps extreme example of this philosophy is that if terrestrial life uses A,T,C,G and U for information storage a Martian organism may use L.M.N.O and P. Again the probability is that life will choose only a few of the possible choices of, in this case, purine and pyrimidine isomers. Therefore, knowing the abiotic reactions are possible in a certain context provides a baseline value from which any anomalous concentrations of organics that may be a 'biosignature' can be detected.

This strategy depends on several key points for implementation.

1) An understanding of possible abiotic chemistry undertaken in Mars environments (including meteoritic infall) and the preservation / diagensis of that signal with time.
2) A clear understanding of the geological context in which measurements are made.
3) A multidisciplinary and multi-measurement approach with convergent data sets from each measurement.
4) Commitment to a null hypothesis that all observations are treated as non-life signatures until a wealth of evidence exists to falsify this hypothesis.
5) Clear operating guidelines and peer review of results and data. It is after all the community and not a single investigator or measurement that will ultimately define a positive "Life Detected" result.

While apparently biased towards the detection of molecular biosignatures, the invocation of a null hypothesis demands similar rigorous examination of data from the detection of possible mineral, isotopic or morphological biosignatures.

**Setting an abiotic signature for Mars**

If, as stated, the detection of life from organic molecules requires an understanding of the abiotic Martian organic inventory then what is our current understanding of Martian organic chemistry. To the first order the answer to this question is that while there are tantalizing glimpses and debate on both landed mission data (Viking) and Mars meteorite data currently there is no uncontested detection of a reduced carbon phase on Mars.

**ALH84001 -** In the debate to understand whether relic Martian life is present in ALH84001, significant research has been conducted to understand the presence and provenance of organic materials, specifically polyaromatic hydrocarbons (PAHs) in this meteorite (Bada et al., 1998; Becker et al., 1999; McKay et al., 1996). Steele et al., (2000) showed the presence of contaminating terrestrial organisms in the meteorite. Carbon isotope analysis ($\delta C^{13}$ and $C^{14}$) shows that there is a high temperature phase of carbon, that comprised approximately 20% of the carbon in the meteorite (~240 ppm), both within the carbonate globules and the host pyroxene of ALH84001 that is indigenous to the meteorite (Jull et al., 1998). Using C-XANES, Flynn et al., (1998), showed the presence of C-C, C=C and possibly C-H bonds in both carbonate and magnetite. Bada et al., (1998) showed that a range of biologic amino acids exist in ALH84001 most, but perhaps not all, of which could be explained by terrestrial contamination. Organic contaminants have been detected ALH84001 (Becker et al., 1999; Jull et al., 1998; Steele et al., 2000; Stephan et al., 2003). Becker et al. (1999) found that only a small proportion (~1%) of the indigenous organic carbon in ALH84001 is accounted for as PAHs and amino acids. The rest is present as a high temperature released macromolecular phase that is postulated to have originated from meteoritic infall to Mars (Becker et al., 1999).

Steele et al., (2008) found polyaromatic macromolecular carbon species (MMC) as well as graphite in intimate association with magnetite in both ALH84001 and terrestrial mantle derived carbonate globules from samples collected from the Svalbard Bockfjorden Volcanic Complex (BVC). The authors demonstrate that MMC synthesis appeared to be associated with known abiotic reactions within the Fe-C-O system and that MMC was produced during precipitation of the carbonate globules and potentially also through thermal decomposition of siderite (Koziol 2004; McCollom, 2003; Treiman, 2003; Zolotov and Shock, 1998; Zolotov and Shock, 2000, Steele et al., 2008). By inference to a terrestrial analogue of mantle origin (BVC), these results appear to represent an explanation of the presence of indigenous organic material in ALH84001 and potentially indicative of an abiotic macromolecular carbon synthesis mechanism on Mars.

**Nakhla -** The presence of organic substances in Nakhla and other meteorites (e.g. polycyclic aromatic hydrocarbons, amino acids and aliphatic hydrocarbons) has previously been demonstrated, suggesting that the abundance of these substances may partially be indigenous and partially a result of contamination from their respective terrestrial environments (Bada *et al*., 1998; Wright *et al*., 1998; Glavin *et al*., 1999,

Flynn *et al*., 1999, Toporski and Steele 2004). Jull *et al*., (2000) argued through $\delta C^{13}$ and $C^{14}$ data that 75% of the carbon inventory in Nakhla was Martian in origin, with terrestrial contamination representing the other 25%. Polycyclic aromatic hydrocarbons, amino acids, and aliphatic hydrocarbons have been detected in Nakhla (Glavin *et al*., 1999; Flynn *et al.*, 1999). Sephton et al., (2002) showed the presence of benzene, toluene, C2 alkyl benzene and benzonitrile in pyrosylates of the Nakhla meteorite that have a carbon isotope distribution similar to the Murchison meteorite. Due to this similarity these compounds are attributed to meteoritic infall to Mars.

Glavin *et al.* (1999) concluded that most of the amino acids in Nakhla were derived from terrestrial sources, probably bacteria. Toporski and Steele (2004) showed the presence of terrestrial organisms throughout a depth profile of the Nakhla meteorite.

**Other Martian meteorites**

Using stepped combustion Grady et al., (2004) showed the presence of a high temperature phase of carbon (released between 600 and 1000°C) that the authors claim is a crystalline carbon phase indigenous to the meteorite. This work follows on from previous observations and represents a consistent data set over 12 Martian meteorites (Wright et al., 1989, 1992; Grady et al., 1997)

**Biotic Carbon**

McKay et al., (1996) postulated that the distribution of polyaromatic hydrocarbons in ALH 84001 was one line of evidence (from 4) that showed possible relic biogenic activity in this meteorite. Since this study, the group has extended the claim of life to Nakhla (within iddingsite that is indigenous to the meteorite), Shergotty and unpublished data on NWA 998 (Gibson et al., 2001).

**Organics Detected on Mars**

Loes Ten Kate (2010) completed an excellent review on the current state of in-situ investigations for Martian organic material. Navarro-Gonzalez (2010) has recently reinterpreted the Viking GCMS detection of chloromethane as organic matter degraded by perchlorate during pyrolysis.

A further interesting aspect of the discovery of iron and potentially stoney meteorites on Mars (Schroeder et al., 2008) is that most iron meteorites consist of a significant proportion of graphitic carbon (Deines and Wickman, 1975). The isotopic signature of which can vary from -4.8 to -24.1 ‰ (Deines and Wickman 1975).

In impact terrains the likely residue of any carbon species in the impactor or impacted terrain is also probably graphitic as evidenced from lunar experience (Steele et al., 2010). However, this does not preclude survival of more volatile species (Ross 2006, Chyba 1989). Separating meteoritic and indigenous Martian carbon may prove challenging for SAM give the similarity of carbon speciation and isotope signature between potential Martian organic material and the Murchison meteorite for example (Sephton et al., 2002). Carbon isotope signals that are used to discriminate terrestrial life are also very similar to that reported for indigenous Martian organic material (Grady et al., 2004 and references therein).

**Conclusion**

With these studies we have a tantalizing glimpse of the processes that could produce organic chemical and life signatures on Mars; abiotic chemistry, meteoritic infall (non indigenous abiotic chemistry) and possibly life. The life detection philosophy outlined in this abstract is robust and has minimal assumptions about the nature of ET life and furthermore, will allow accurate deconvolution of different carbon pools (abiotic V biotic etc). While the Mars program and community regard the finding of life as of paramount importance, the lack of life on Mars would beg the question "why ?" and what is unique about the earth to allow life to start here. N would equal 2. Therefore the search for life on Mars is a search for our own beginnings.

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**Characterizing terrestrial samples with pyrolysis-GC-MS similar to MOMA aboard ExoMars-2018.**

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**Introduction:** The ExoMars-2018 rover mission will combine both in-situ analysis of Martian (sub) surface material and caching of samples to be returned to Earth at a later time. The Mars Organic Molecule Analyzer (MOMA) aboard the rover shall characterize the organic compounds in these samples and thereby support the sample selection process for caching. MOMA will use a modified pyrolysis technique (similar to the SAM instrument of Mars Science Laboratory, MSL) next to Laser Desorption and Ionization (LDI) coupled to a Mass Spectrometer (MS). The data obtained by MOMA will be a key indicator if the sample is worth of sample return.

Pyrolysis-GC-MS (Gas Chromatography - Mass Spectrometery) is one of the principal operational modes of the instrument. This mode does not require any sample preparation except crushing. In geochemistry pyrolysis-GC-MS is used for the analysis of kerogenes, while the more volatile materials are solvent-extracted (rather than heated) and analyzed by GC-MS. Here we present pyrolysis-GC-MS data acquired both by a commercial setup and an early MOMA prototype, in order to demonstrate, how MOMA will be able to support the sample selection for caching.

**Experiments:** The samples described here were collected during the joint ESA-NASA Arctic Mars Analogue Svalbard Expedition AMASE 2011 [1]: (a) Coletthøgda, Butterfinger (float, largely calcite, some dolomite and quartz, possibly bioherm), and (b) Coletthödga, Kit Kat (float, mainly dolomite with some quartz, possibly from stratified part of outcrop below Butterfinger). The samples were ground in a mortar, placed onto a small platinum filament and pyrolysed at temperatures above 800°C in a Pyrola pyrolysis unit. The evolved gas was analyzed with a Varian 4000 GC-MS. Several of the samples contain only minute amounts of organics and therefore no GC-MS data could be acquired. However, the more organic rich ones contain always benzene, toluene and some other simple organic molecules. The resulting GC-MS plots are complex. However, their analysis can be simplified by selecting some specific molecular masses.

**Results:** Patterns in the distribution of long-chain hydrocarbons provide information on the origin and geochemical evolution of the organics in the sample.The two samples show very significant differences in long-chain hydrocarbon distribution (Figs. 1 and 2)

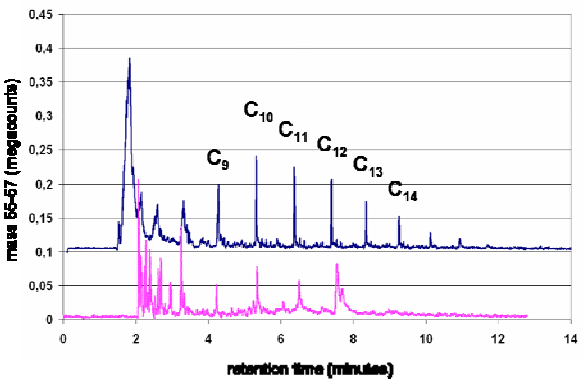


**Figure 1:** Pattern of long-chain hydrocarbons in the GC-MS plot of the Butterfinger sample. Upper plot (blue) shows data obtained in commercial setup, lower plot (pink) shows experimental data obtained by a flight-like breadboard of MOMA. Selected masses: 55-57 u.

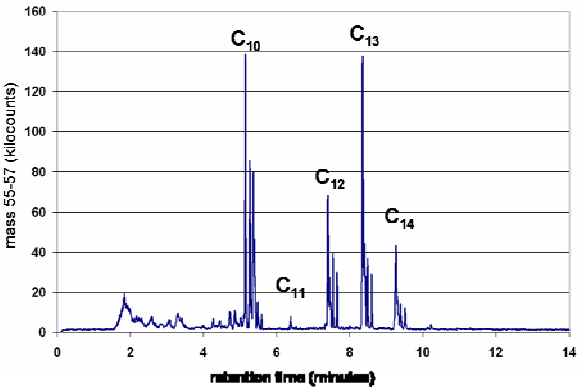


**Figure 2:** Pattern of long-chain hydrocarbons in the GC-MS plot of the Kit Kat sample. Selected masses:55-57 u.

The a flight-likebreadboard of MOMA and the commercial Pyrola/Varian instrument give similar results, although the larger masses are absent due to temperature limitations of the breadboard (Fig. 1).

MOMA has two further operational modes: (1) derivatization GC-MS and (2) Laser Desorption MS. Both modes widen the field of view of the instrument but the current status of the instrument's breadboard does not include these two modes.

**References:** [1] Steele, A. et al. (2010), *LPS XLI*, Abstract #2398.

**Single Cell Genomics: Reaching the Limits of Life Detection Sensitivity.** R. Stepanauskas, Bigelow Laboratory for Ocean Sciences (McKown Point 180, West Boothbay Harbor, ME 04575).

**Abstract:** Reading genetic information encoded in individual, uncultured microbial cells represents the ultimate level of life detection sensitivity and genetic data richness. It also allows, for the first time, the study of multiple genes and entire genomes of uncultured microorganisms, independent of the complexity of their communities and compatible with extremely low sample quantities.

Single cell genomics relies on the physical separation of individual cells, followed by their lysis, whole genome amplification, and subsequent DNA sequencing. In 2009, Bigelow Laboratory for Ocean Sciences established the first high-throughput facility in this field, providing single cell genomics services to the broad scientific community. During its first two years in operation, Bigelow Laboratory Single Cell Genomics Center contributed to cutting-edge research projects at over 30 organizations around the globe. Over 300,000 individual cells have been analyzed by the Center so far, providing unique access to genomic DNA, without cultivation biases, from microorganisms representing over 60 phyla of bacteria, archaea and protists. The types of samples processed by the Center range from marine to deep subsurface to mammalian gut content, and the type of research questions addressed range from microbial ecology and evolution to human health and bioprospecting. Center's research accomplishments include discoveries of inorganic carbon fixation pathways in abundant bacterial groups in the dark ocean, in situ trophic interactions of uncultured protists, identification of novel phototrophs, etc. [1-10]. An example of exobiology-relevant results include genomic sequences representing multiple microbial candidate divisions from a subsurface sample collected at the Deep Underground Science and Education Laboratory (DUSEL) (Fig. 1). Our findings of bacteria-like Sigma factors and RuBisCO genes in some of the discovered novel archaeal lineages provide new insights into the early evolution of life on Earth and indicate sources of energy utilized by subsurface microorganisms.

In summary, single cell genomics is no longer a theoretical possibility but rather a rapidly expanding research field that offers unprecedented opportunities for the search of extraterrestrial life at the ultimate level of sensitivity – single cell.

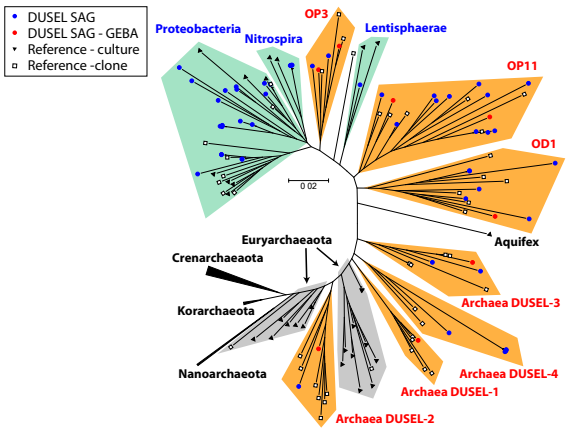


**Figure 1.** Phylogenetic composition of microbial single amplified genomes (SAGs) recovered from the Deep Underground Science and Education Laboratory (DUSEL).

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**ULTRASENSITIVE DETECTION OF TERRAN DNA FOR PLANETARY PROTECTION.** T. A. Stephenson[1], A. S. Burton[2], N. Lehman[3] and J. P. Dworkin[4], [1]Code 541 Materials Engineering Laboratory NASA/GSFC, Greenbelt MD 20771, [2]Code 691 Astrochemistry Laboratory (ORAU) NASA/GSFC, Greenbelt MD 20771, [3]Department of Chemistry, Portland State University, Portland OR 97201, [4]Code 691 Astrochemistry Laboratory NASA/GSFC, Greenbelt MD 20771

**Introduction:** There is a critical need to rapidly detect, classify, and enumerate the widest possible spectrum of Earth microbes carried by spacecraft. These may inhabit surfaces and bulk materials of spacecraft at low densities before, during and after assembly and launch processing. The assay method used currently is based on colony counts of spore-forming organisms obtained by wiping surfaces with cotton swabs. Isolated organisms are cultured for 72 hours, making this a labor-intensive process with a relatively long turn-around time. It is also limited to culturable bacteria (<1% of bacteria [1]). Two other assays are in development: Limulus Amebocyte Lysate (LAL) and ATP bioluminescence [2]. These methods both offer high sensitivity (10 cells / mL and 5 cells / mL, respectively) but also have significant limitations. In the case of LAL, only gram-negative bacteria and fungi can be assayed, while ATP bioluminescence requires viable cells and must be calibrated for individual organisms [3]. Finally, neither of these assay methods can provide information about the type of organism being detected.

Recent advances in DNA amplification offer a way to further increase the sensitivity of contaminant assays while also providing information about the source of the contamination. Multiple displacement amplification (MDA) is an extremely sensitive technique originally developed to amplify the DNA from a single cell for sequencing. As such, MDA is ~10,000-fold more sensitive than the Polymerase Chain Reaction (PCR) for detecting and amplifying DNA from environmental samples [4]. Furthermore, because MDA copies the entire genome of an organism, MDA does not suffer the same primer restrictions as PCR amplification and can be accomplished on the same time-scale. In addition, products of MDA are templates for PCR, allowing the species of contaminating organisms to be obtained. Through single cell detection, the potential to identify the type(s) of contaminating organism(s), and a processing time of hours, DNA detection by MDA represents a major advance over existing and developing planetary protection technologies.

**Background:** The driving force for the development of the MDA process was a desire to assess the biodiversity of the microbial world. This was previously done by PCR. However, natural samples often contain only low levels of DNA as well as ions and molecules that inhibit PCR, limiting the success of this approach. Because PCR depends on the use of specific primer pairs, this technique requires that you know what DNA you are trying to amplify. A common target are ribosomal RNA (rRNA) genes; however, these differ between types of organisms and require different primers. (*e.g.,* eukaryotic 18S rRNA or bacterial 16S rRNA genes). The bottom line is that PCR applied directly to soil samples has a detection limit of $10^4$ cells [5]. This level of sensitivity will show inherent bias towards the most abundant organisms in a sample, and exclude rare bacteria. Thus, a technique was required that would make multiple copies of any DNA present. Non-specific DNA amplification by the MDA technique was developed at Molecular Staging, Inc., by Lasken [6]. This method can amplify dilute quantities of DNA and can generate over a billion copies starting from a single molecule of DNA [7-9]. In addition, MDA has been shown to be less susceptible than PCR to inhibitors such as humic acids and exopolysaccharides that are commonly found in environmental samples that block DNA amplification reactions [10]. Thus, MDA is now widely accepted in the genomics field as the preferred method to amplify environmental samples including soil [11-13], deep mine environments that have very low biomass [14-15] and clinical samples [16].

In the laboratory this technique has demonstrated detection sensitivity to 1 fg ($1 \times 10^{-15}$ g) of DNA in 1mL water [17]. There are ~2.5 fg of DNA in a typical bacterium, so this technique demonstrates the detection of a DNA fragment and therefore, part of a DNA molecule from a single cell. By enabling the detection of a single organism, MDA theoretically represents the most sensitive method currently available to the Planetary Protection Program. In addition, because MDA is not targeted to specific regions of DNA, it can detect: viable and culturable microbes, viable but non-culturable microbes, non-viable microbes, molecular fragments of microbes, and DNA-based viruses, in addition to the DNA of higher organisms that are involved in spacecraft construction intentionally (engineers) or inadvertently (*e.g.,* mice). We have been successful using MDA to detect rare sequences in environmental samples.

**Approach:** This approach represents a major improvement over existing Planetary Protection (PP)

techniques summarized in Table 1. Using optimized extraction techniques together with MDA and PCR methods enables evaluation of the biological cleanliness of NASA spacecraft and the biological cleanliness of returned samples.

**Table 1 – Comparison of PP Detection Techniques**

| Technique | Limit of detection (cells/mL) | Notes |
|---|---|---|
| NASA Standard Assay (Spore Culture) [18] | Single cell on agar | Heritage use; applicable only to culturable cells (<1% of currently known bacteria) |
| LAL assay [19] | ~10 | Sensitive to living and dead cells. Limited to Gram negative bacteria and fungi. |
| ATP bioluminescence [20] | ~5 | Sensitive to living and dead cells. However, ATP in dead cells quickly degrades. |
| PCR [21] | ~10,000 | Amplification of target DNA sequences only. Reaction easily inhibited in environmental samples. |
| MDA[22] | <1 | General amplification, detection of ALL Terran DNA. Enables PCR of low biomass environmental samples. |

By combining the high sensitivity and general amplification abilities of MDA with the specificity obtained with specific primers in PCR, we meet the Planetary Protection goals of enhanced sensitivity and broadened diversity in the detection of Earth microbes.

In summary, we will be able to detect, classify and enumerate both outbound biological contamination and also returned biological contamination for the Earth-like bioburdan that makes the round-trip in sample-return missions.

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**Microbial Detection at Low Levels by [125]I Radiolabeling.** D. P. Summers[1] and H. K. Kagawa[2], [1]Carl Sagan Center, SETI Institute (c/o NASA Ames Research Center, MS 239-4, Moffett Field, CA, David.P.Summers@nasa.gov), 2Carl Sagan Center, SETI Institute (c/o NASA Ames Research Center, MS 239-15, Moffett Field, CA).

**Introduction:** It has long been acknowledged that culturing is an inadequate method for the detection of microorganisms. Modern methods that detect the presence of life typically rely on detection of the presence of a type of molecule present in life. Two common modern methods are the LAL (Limulus Amebocyte Lysate) test, which relies on endotoxins present in certain types of bacteria, the ATP (Adenosine Triphosphate) assay, and PCR (polymerase chain reaction), which involves the detection and amplification of DNA. We are working on a method that has potential advantages over these, the detection of microbial contamination by the detection of the organism's proteins. This is accomplished by the labeling of these proteins with a radioactive label, $^{125}$I.

**Detection by $^{125}$I Labeling:** Detection of proteins as signatures for the presence of organisms only requires the most general of assumption, that the organism uses proteins. All known organisms, across all three domains of life, use proteins. Another advantage of using proteins as a signature for life is that the large number of proteins contained in a cell makes detection easier, allowing the detection of very small numbers of organisms. An average *E. coli* is over 50% protein by dry weight and contains millions of protein molecules [1]. (50% by dry weight or 15% by volume is a good rule of thumb for most cells). The large size of proteins means that they will have a number of tyrosine residues (the easiest residue to label), allowing for multiple labels to be attached to each molecule, further multiplying sensitivity.

What are our expected detection limits for protein radiolabeling analysis? For ordinary detection of $^{125}$I, 30 counts per second is fairly typical and 10 counts per second is reasonably achievable. This also results in measurement times (to achieve acceptable statistics) of seconds, which is more than fast enough for convenient analysis. If one assumes 30 cps and a detector efficiency of 75%, that corresponds to 40 disintegrations per second. A half life of 60 days correspond to a first order rate constant of $1.3 \times 10^{-7}$ sec$^{-1}$. One would thus need $3 \times 10^{8}$ labels to give 30 cps. An average *E. coli* bacteria has $2.3 \times 10^{6}$ protein molecules per cell [1]. Assuming that one is able to place only one label per protein, one would be able to detect on the order of 130 cells. This is a fairly conservative estimate since most proteins will have multiple tyrosine residues and one could easily get below 30 cps. A typical protein might have 10-20 tyrosine residues, allowing the detection limits to potentially be cut by and order of magnitude. Multiple labeling and a well shielded counter (with a 10 cps or lower background) could detect less than 10 cells.

Radiolabeling techniques are inherently sensitive and $^{125}$I benefits from a 60 day half-life, providing greater activity and signal per unit number of labels. Additional sensitivity can be obtained by use of a Multiphoton Detection (MPD). By taking advantage of selected isotopes, that decay by the emission of multiple photons, MPD can use coincidence methods to screen out many background events and detect radioisotopes, such as $^{125}$I, at below background levels. This can enable thousand-fold improvements in sensitivity. That would make single cell detection easy to achieve. Even spores will have ample number of proteins for single cell detection.

The detection of live cells is accomplished by detecting the proteins that are released upon cell lysis by…

1) Separation of cells/spores from background proteins.

2) Lysis of cells.

3) Labeling of released proteins & separation from unreacted label.

4) Detection of radiolabeled proteins.

**Comparison to Other Methods:** This method has advantages in both generality and sensitivity. The LAL test will only detect organisms that produce endotoxins [2, 3]. The detection limits of organisms in the LAL test is about $10^{4}$ cells/ml [2].

The ATP test is a fairly general test, all known organisms use ATP [4-6]. However, the amount of ATP is more variable between organisms (ATP is only present in trace amounts in spores). In any case, the number of proteins in a cell is much higher than the number of ATP molecules. The ATP test can detect $10^{3}$ cells, or $10^{4}$ cells/ml, of "average" bacteria [6]. (For cells with very low levels of ATP, such as spores, sensitivities will be correspondingly worse.)

PCR techniques certainly have the potential for high sensitivity, though they require much more time and technique than the other methods. Significant issues revolve around the requirement for primers, as even general or "universal" primer sets most likely can detect only ~80% of known organisms. It is unlikely

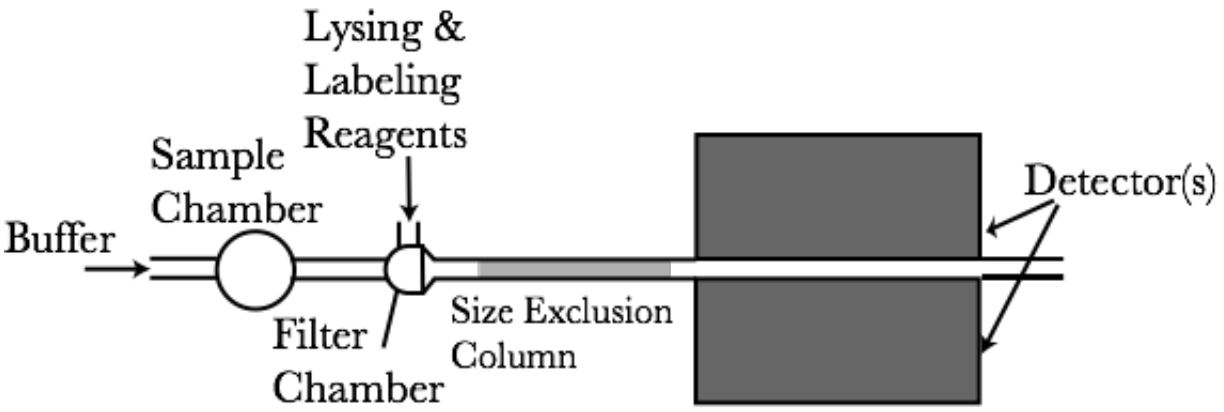


Figure 1. Schematic of flow system for analysis.

to be able to detect unknown organisms in extraterrestrial samples.

**Current work:** We are currently working on develop of this method through a grant from NASA's planetary protection program. There we seek to detect contamination of Earth organisms on spacecraft. Recent attention has been toward methods of universally lysing organisms in ways that are compatible development of a fast and automated analysis method. The best results were obtained by the use of XS (potassium ethyl xanthogenate) buffer, used for opening Halococcus (1% XS, 20 mM EDTA, 1% SDS, 800 mM ammonium acetate, 100 mM Tris-HCl, pH 7.5, incubated at 60 °C for 2 hr, Vortex for 10 sec, on ice for 10 min.)

This seems to allow good lysis while not requiring extensive handling steps, allowing it to be applied easily between cell separation and protein labeling.

While it is being developed for detection of Earth organisms on spacecraft, this method is very suitable for use with extraterrestrial samples. For samples of extraterrestrial origin, with unknown organisms, a method of high generality is needed. This method makes only the most general assumptions (that organisms use proteins). This makes it very applicable to the analysis of extraterrestrial samples. The durability of proteins (especially since there is no requirement to maintain enzymatic function) means that no significant restrictions are imposed upon sample handling and storage.

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**CHIRAL LIFE DETECTION .** H. J. Sun, Desert Research Institute, 755 E. Flamingo Road, Las Vegas, NV 89119. henry.sun@dri.edu

One of the outstanding questions in the search for life on Mars concerns the nature of the Viking labeled release experiment (LR). The result of the early *in situ* experiment was consistent with the presence of heterotrophic metabolism on Mars. A mixture of sugars, lactic acid, and amino acids that were added to soil in an aqueous solution were rapidly oxidized to carbon dioxide (Levin and Straat 1976). The LR is controversial, however, because it could not rule out the possibility of an inorganic chemical reaction with soil oxidants. Recently, there has been some interest in the development of a chiral LR that could be implemented on Mars to resolve the debate. Instead of a racemic mixture, a chiral LR would use pure enantiomeric preparations. Biological activity is expected to be selective, destroying only one of the two enantiomers, whereas chemical reactions would destroy both.

I present the results of a comprehensive test of the chiral life detection concep t using terrestrial microorganisms. Tested compounds include sugars, lactic acid, and amino acids. The results indicate that biological selectivity is not absolute. Lactic acid and amino acids can conve rt from one enantiomeric form to another by racemization. Many Earth microorganisms possess racemases that facilitate the conve rsion. As a result, they can metabolize the non-traditional enantiomer (L-lactate and D-amino acids) if given sufficient time to express the relevant racemases (Figure 1) (Moazeni, Zhang, and Sun 2010).

Sugars do not racemize. Also, they are brought into the cell by chirally sensitive transport proteins. As a result, microorganisms recognize or prefer the enantiomeric forms that occur naturally. Some sugars, such as glucose and xylose, occur naturally only in D-form. Soil microorganisms utilize these sugars only if they are provided in D-form (Figure 2) (Sun et al. 2009). Other sugars, such as fucose and arabinose occur primarily in L-form. Correspondingly, heterotrophic bacteria and fungi prefer these sugars in L-form.

The situation with sugars is complicated by cross reaction. Two different sugars can be structurally so similar that they could be transported and metabolized by the same enzymes. For example, bacteria that grow on D-arabinose also utilize L-fucose, and vice versa, without prior exposure.

This complexity and variability does not invalidate the concept of chiral LR. To the contrary, it adds strength. For example, if a planetary sample prefers one sugar in D-form and another sugar in L-form, it is difficult to argue that it could be due to anything other than life. Nor does the presence of amino acid racemases create a serious problem. It only means that it is important to collect the result early, before the racemase enzymes have a chance to be expressed.

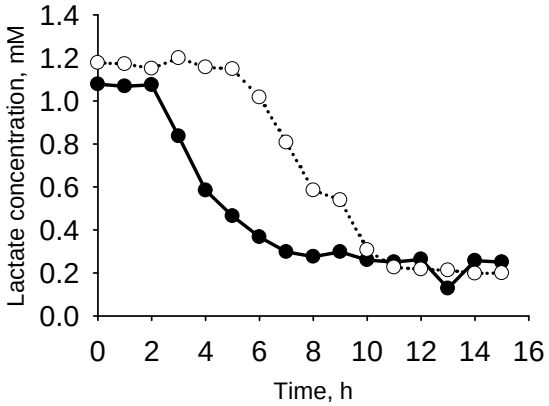


**Figure 1. Consumption of D-lactate (closed symbol) and L-lactate (open symbol) added to a soil from the Atacama Desert.**

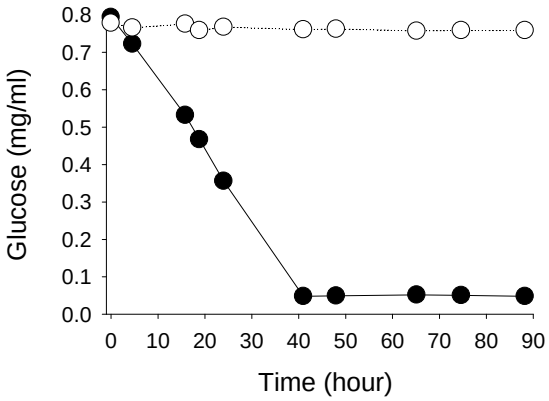


**Figure 2. Consumption of D-glucose (filled symbol) and L-glucose (open symbol) added to a culture of *Penicilium expansum*.**

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**NEW INSIGHTS INTO THE ORIGIN OF MAGNETITE CRYSTALS IN ALH84001 CARBONATE DISKS**

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**Introduction:** Martian meteorite ALH84001 preserves evidence of interaction with aqueous fluids while on Mars in the form of microscopic carbonate disks believed to have formed ~3.9 Ga ago at beginning of the Noachian epoch. Intimately associated within and throughout these carbonate disks are nanocrystal magnetites ($Fe_3O_4$) with unusual chemical and physical properties. The origin(s) of these magnetites has(have) become the source of considerable debate. One group of hypotheses argues that these magnetites are the product of partial thermal decomposition of the host carbonate; this decomposition event being separately argued to have occurred under the mutually exclusive conditions of kinetic or pseudo-equilibrium control. Alternatively, we have argued that the origins of magnetite and carbonate are unrelated; that is, from the perspective of the carbonate the magnetite is allochthonous. This hypothesis is based on two lines of research: (1) the comprehensive, detailed characterized of the compositional and structural relationships between the carbonate disks, their associated magnetites and the orthopyroxene matrix in which they are embedded [1]; and, (2) the results of experimental thermal decomposition studies of sideritic carbonates, conducted under a range of heating scenarios, which have repeatedly failed to produce magnetite nanocrystals with the chemical and physical properties of those present in the ALH84001 carbonate disks.

**Methods:** Seven focused ion beam (FIB) transverse sections were extracted from two carbonate disks -- three spanning the inner disk cores and four from the thin rims surround the cores. Extracted FIB sections were either *Pt*-welded *in situ* onto a *Cu* TEM crescent lift-out grid, or placed *ex situ* onto a continuous *C* film *Cu* TEM grid. Sections were analyzed by high-resolution transmission electron microscopy (HRTEM) equipped with light element energy dispersive X-ray spectroscopy (EDX).

To investigate the chemical and physical properties of magnetite formed through thermal decomposition, a sample of Roxbury siderite ($(Fe_{0.84}Mg_{0.10}Mn_{0.04}Ca_{0.02})CO_3$ [2]) was used as a standard. This was decomposed using two different heating regimes; 'slow' ($\frac{\partial T}{\partial t}$ ~ $10^{-2}$ K·sec$^{-1}$) and 'fast' ($\frac{\partial T}{\partial t}$ ~ $10^{8}$-$10^{9}$ K·sec$^{-1}$) to produce magnetites formed under both kinetic and thermodynamic control. An unheated sample served as a control and all samples were embedded in epoxy and sectioned for analysis by diamond knife ultramicrotomy.

**Results:** *ALH84001*. Carbonate disks can be envisioned as being composed of three concentric annular zones. Moving out from the center there is an inner and outer central core which is itself surrounded by a thin rim composed of optically alternating black-white-black layers. ALH84001 magnetites are embedded within all compositions of carbonate, including the *Fe*-free magnesite ($MgCO_3$) present in the white layer of the outer rim (Fig. 1). The highest density of magnetites occurs within the two black layers of the outer rim. The majority of magnetites are stoichiometrically pure $Fe_3O_4$ although there were several notable exceptions, e.g., a small fraction of magnetites contain minor to trace amounts of *Cr*, which was undetectable in the surrounding host carbonate (Fig.2).

*Roxbury Siderite Control Sample*. The bulk composition of Roxbury siderite is analogous to the most *Fe*-rich component of the ALH84001 carbonate disks ($(Fe_{0.75}Mg_{0.24}Mn_{0.003}Ca_{0.004})CO_3$ [3]. However, TEM characterization at the sub-micron scale reveals significant variations in *Fe*:*Mg* ratio relative to the bulk composition and appeared to be correlated with grain size. The fine grain (<100 nm) component is *Mg*-poor (*Fe*:*Mg* > 20:1) while, the coarse grained component is relatively *Mg*-rich (*Fe*:*Mg* < 8:1). In both size ranges *Mn* and *Ca* are uniform in comparison.

*Roxbury Siderite 'Fast' and 'Slow' Heated Samples*. Under both 'fast' and 'slow' heating regimes, decomposition of Roxbury siderite resulted in the formation of impure ferrites with the with variable *Mg* and a relatively invariant *Mn* content. (Fig. 3). Since the *Mg* and *Mn* variation mirrors that of the unheated carbonate this variation is simply a reflection of the initial content of the carbonate. Notably, in neither the 'fast' or 'slow' products did we find any evidence for discrete *MgO* or *CaO* phases.

These results, in conjunction with prior carbonate decomposition studies of the *Fe*-, *Mg*- and *Ca*-ternary carbonates (*e.g.*, [4,5] and references in [1]), demonstrate that the decomposition of impure, cation substituted siderites invariably yields impure *Fe*-oxides.

**Discussion & Implications:** Thermal decomposition of Roxbury siderite under <u>both</u> 'fast' and 'slow' heating resulted in the formation of impure (*Mg*,*Ca*,*Mn*)-ferrites. These findings are in agreement with prior decomposition studies performed under a wide variety of conditions and the thermal

decomposition of mixed cation siderite can be summarized as:

$$3(Fe_xMg_yCa_zMn_{[1-x-y-z]})CO_3 \xrightarrow{\Delta}$$

While the thermal decomposition hypotheses for the origin of magnetites in ALH84001 carbonates is appealing in its simplicity it is nonetheless inapplicable since magnetites formed this way fail to show the chemical and physical properties characteristic of magnetites we actually do observe in ALH84001 carbonate disks. For example, it is difficult to suggest a process by which the magnesite, which is essentially *Fe*-free, decomposed to form magnetite. Furthermore, it would be difficult to explain the presence of chemically impure magnetites with minor to trace amounts of *Cr* since this element cannot substitute into the trigonal ($R\bar{3}c$) structure of carbonate.

We argue that the majority of ALH84001 magnetites has an allochthonous origin, that is they were incorporated from an outside source in to the carbonate. This origin does not exclude the possibility of formation by biogenic processes, as has been proposed in previous studies.

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**Fig.1**. *Left*: TEM view of ALH84001 magnetites (arrows) embedded within the magnesite layer. *Right*: EDX spectra for one of the magnetites (red circle), the surrounding magnesite matrix (blue circle), and the difference spectrum (green). They show the host matrix is essentially *Fe*-free while the magnetite is *Mg*-free. The presence of these magnetites embedded in the magnesite band indicates they could not have formed by thermal decomposition of the magnesite matrix.

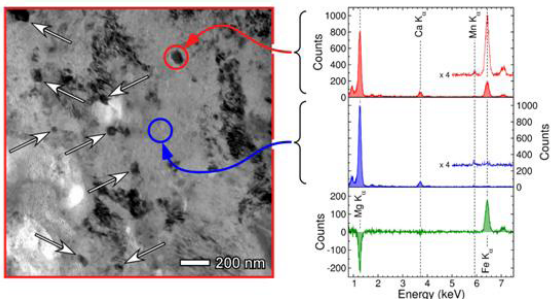



**Fig.2**. *Top views*: FIB section extracted from the core-rim interface of an ALH84001 carbonate disk (left) and high magnification of magnetite crystals within that section (right). *Bottom views*: Element maps for *Fe* (left) and *Cr* (right) of the largest magnetite in the field of view (top right). This magnetite has a composition of *Fe* ~70.1 wt.%, *Cr* ~ 2.3 wt.%, and *O* ~27.6 wt.%, corresponding to a stoichiometry of $(Fe_{2.9}Cr_{0.1})O_4$ (*i.e.*, ~ 3.3% $Cr_2O_3$).

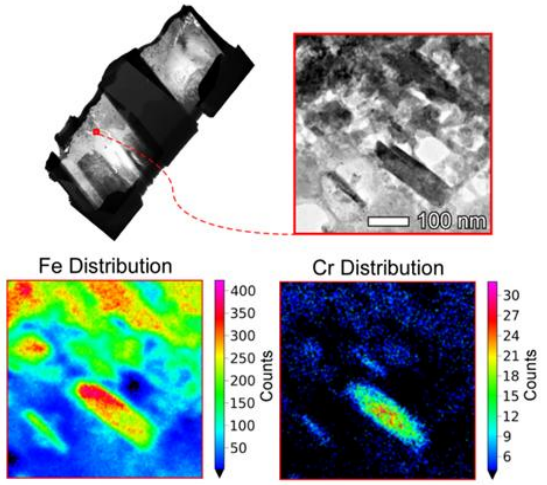
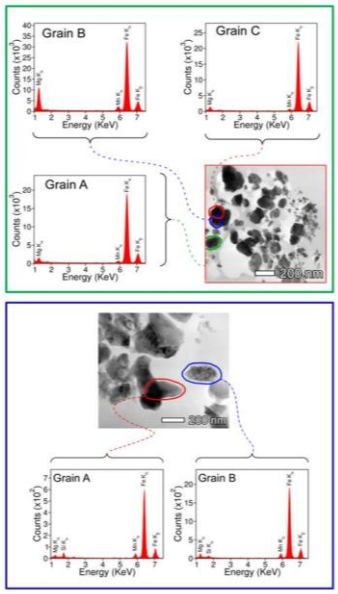



**Fig. 3**. EDX spectra and TEM views of magnetites formed from the 'slow' (green box) and 'fast' (blue box) heating of Roxbury siderite. Although spatially associated, variations in *Mg* content are apparent while *Mn* content is relatively uniform. All magnetites formed from the thermal decomposition of Roxbury siderite contained chemical impurities reflecting the composition of the precursor Roxbury siderite.



# A Miniature AOTF-LDTOF Spectrometer Suite for the Detection of Biomarkers on Planetary Surfaces.

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We discuss the development of a miniature near-infrared point spectrometer, operating between 1.6–3.5 $\mu$m, based on acousto-optic tunable filter (AOTF) technology. This instrument may be used to screen and corroborate analyses of samples containing organic biomarkers or mineralogical signatures suggestive of extant or extinct organic material collected *in situ* on planetary surfaces. The AOTF point spectrometer will be paired with a laser desorption time-of-flight (LDTOF) mass spectrometer and will prescreen samples for evidence of volatile or refractory organics before the more power intensive laser desorption and subsequent mass spectrometer measurements. We present laboratory analysis of geological samples of known astrobiological importance, with and without organic biomarkers.

**Introduction** On future surface missions to Mars, small bodies, and outer solar system satellites, robust sample screening and selection will be essential to achieve the maximum scientific benefit within limited payload resources. On both in situ and sample return missions, a common central goal will likely be to understand the production and processing of organic molecules in the solar system and their relationship to prebiotic chemistry and habitable environments.

The samples selected for sophisticated laboratory analysis must be carefully vetted by analytical tools that provide the greatest assurance of science value. One approach is the identification of key organic functional groups by a spectroscopic prescreening tool, followed by organic compound analysis with one or more mass spectrometric methods of increasing complexity.

The addition of an AOTF spectrometer to an existing LDTOF instrument will enable significant diagnostic capability without exceeding the resources of a small mobile laboratory, resulting in a powerful tool for astrobiological exploration of planetary surfaces in our solar system.

**Technology Development** AOTF systems provide great flexibility, being very compact and electronically programmable, with time-averaged power requirements of a few watts or less. They can provide an arbitrary spectral selection over a wide tuning range by utilizing a birefringent $TeO_2$ crystal, which acts as a diffraction grating when compressed using RF waves. With broadband light as an input, orthogonally polarized, spectrally narrow beams are diffracted within the crystal and can be separately re-imaged at the output. The AOTF material, $TeO_2$, is inherently rugged and radiation hard. Furthermore, these devices have no moving parts, making them an attractive option for space flight. Our group has a demonstrated history of developing and using AOTF imaging spectrometers for planetary science applications [1, 2, 3, 4, 5].

The LDTOF mass spectrometer provides pulsed-laser desorption and analysis of refractory organic compounds up to >5,000 Da on a spatial scale of 50-100 $\mu$m determined by the laser spot size at the target. At higher laser power, it also measures major, minor, and trace elements with parts-per-million sensitivity. The LDTOF employs a pulsed 355 nm Nd:YAG laser to desorb and ionize analyte from a solid surface. It collects laser-desorbed ions by drawing them from the sample surface into the ion extraction lens. The ions are focused into a time-of-flight analyzer and terminate at a microchannel plate detector and anode. The voltage pulses are then acquired as a function of time on an oscilloscope. This instrument has been described in detail previously [6].

The AOTF and LDTOF spectrometers have similar requirements for precise positioning of sensor elements near the sample surface. Using a shared optomechanical design, we realized significant savings in instrument mass and complexity. Concepts for sample acquisition and manipulation to permit vacuum analysis on bodies with substantial atmospheres (e.g. Mars, Titan) are under separate development and will be tested with the AOTF-LDTOF in the future.

**Laboratory Measurements** Spectral detection of biological materials on mineral surfaces first requires a thorough characterization of the uninhabited host minerals, measured using the same instrument. We acquired near-IR spectra of a collection of field samples using both the AOTF spectrometer breadboard and the LDTOF spectrometer. The sample suite includes evaporites (sulfates, carbonates), clays, and iron oxides, all of which can be linked to aqueous environments and are therefore of high astrobiological interest.

We also used both instruments to record the spectrum

of a black desert varnish coating on a fractured sample of weathered rock obtained at the Luis Lopez mine site near Socorro, NM. The uncoated side appeared to have been fractured from a larger rock, and thus has a shorter exposure age. The dark color of the desert varnish layers results from a high concentration of oxidized manganese, which can result from either biotic or abiotic processes. However, strong evidence suggests that oxidized Mn in rock varnish is produced by mixotrophic microorganisms in locations that lack abundant organic acids [7]. The strong contrast in spectral reflectance measured between these two samples parallels potential spectral measurements of geologic samples on other planetary surfaces. The ability to recognize these spectral differences in samples within geographic proximity as a biotic precipitate will be essential to determine the importance of follow-up measurements.

Finally, we used both the AOTF and LDTOF instruments to measure basalt samples that were both "neat" and doped with pyrene, a polycyclic aromatic hydrocarbon, in order to determine whether we could detect the presence of hydrocarbons in the rock. We see differences in the spectra between the neat and doped basalts, which we are investigating further.

**Summary and Conclusions** Our instrument development efforts to date have focused on two parallel efforts: the assembly, characterization and demonstration of the compact AOTF spectrometer, and the modifications to the LDTOF chamber in order to accommodate it. The design challenges associated with both aspects of this effort are being successfully addressed, and both the AOTF and the LDTOF are now being used to measure reference samples in the laboratory.

The AOTF measurements of sample reference spectra show that the wavelength calibration of the instrument is very accurate, permitting the identification of known spectral features. The LDTOF mass spectrometer measurements show the expected major and minor elements present in Mars analog samples that have been independently verified with an x-ray diffraction instrument. A comparison between the AOTF and LDTOF measurements of dolomitic samples reveals the complementary nature of the two data sets. The LDTOF identifies the Ca and Mg elemental constituents deriving from the dolomite component of the rock sample. Independently, on a mineralogically related (but not identical) sample, the AOTF spectrometer clearly reveals carbonate peaks. Together, these data sets are consistent with the presence of dolomite. In the near term, we plan to conduct LDTOF measurements on the same sample set used in AOTF studies to fully characterize a common set of minerals. To this, we will add a complement of astrobiologically relevant biosignatures from a variety of geomicrobial study sites well characterized in our previous work [8, 9, 10, 11, 12, 13]. These include desert varnish on volcanic, sedimentary, and igneous bedrock, gypsum weathering rind and evaporite communities, travertines and tufas, and a spectrum of biofabrics and biominerals from cave deposits and surfaces.

**Acknowledgements** This work was supported by grants from NASA's EPSCoR and ASTID programs through award numbers NNX08AV85A and NNX08AY44G.

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**TERRESTRIAL WEATHERING OF CHONDRITES IN NATURE AND CONTINUING DURING LABORATORY STORAGE AND PROCESSING: REVIEW AND IMPLICATIONS FOR SAMPLE INTEGRITY.** Michael A. Velbel, Department of Geological Sciences, 206 Natural Science Building, Michigan State University, East Lansing, MI, 48824-1115 (velbel@msu.edu).

**Introduction:** Meteorites are naturally delivered samples from a variety of parent bodies throughout the solar system. Soluble mineral products of aqueous alteration and organic-chemical compounds occur in some chondritic meteorites and provide evidence for various aspects of the presence and nature of water and possible prebiotic chemistry at different episodes in Solar System evolution. However, these same minerals are highly vulnerable to modifying processes upon arrival at Earth. Some are so reactive in the presence of water (even as vapor) that even exposure to water in ostensibly dry environments (including laboratory atmosphere) results in elemental mobilization and formation of secondary minerals (usually evaporites) [1-11].

Chondrites, from small primitive, undifferentiated asteroidal parent bodies, have been shown to have been affected by redistribution of soluble minerals after recovery, during curatorial storage and processing. Proper planning for sample integrity and proper anticipation of the effects of inevitable exposure to Earth's atmosphere during storage and examination in terrestrial analytical laboratories both require thorough understanding of how minerals like those expected in returned samples from primitive small bodies react with terrestrial moisture and oxidants. This presentation reviews published accounts of the response of chondritic minerals to the moisture and oxidants in the terrestrial environment, and briefly explores their implications for sample return.

**Terrestrial weathering of ordinary chondrites:**

*Natural.* Terrestrial weathering of ordinary chondrites (OCs) is a natural experiment on how OC minerals respond to low-temperature, low-water-rock-ratio aqueous alteration. Oxidation of Fe in metal, sulfides, and ferrous silicates is ubiquitous in naturally weathered OC finds [1-6], in falls subjected to even a few decades of hot-desert weathering [6], and in OC falls recovered and curated promptly after recovery [6]. Slow hydrolitic weathering of primary OC silicates (olivine, pyroxene) concurrent with rapid Fe oxidation in metal and troilite liberates Mg, Ca, and Si [6,7]. Some Mg, Ca, and Si liberated from OC silicates by weathering are incorporated into "rusty" and silicate-mineral products [1-6] and / or mobilized and incorporated into sulfate and carbonate products of weathering [3,7-10]. Some Mg, Ca, and Si liberated from OC silicates by weathering are lost (leached away) during terrestrial weathering of ordinary chondrites [6].

*Post-recovery, laboratory processing and storage.* All oxidation, hydrolysis, hydration, and product-forming phenomena documented to affect OC finds in nature have been documented to continue in OC samples during processing, storage and examination in curatorial and laboratory settings, producing mineralogical and textural effects at scales of tens of microns easily discernable at TEM and even SEM magnifications on timescales of decades or less [6]. For example, modification of the OC find LEW 85320 has been shown to involve hydrolysis of olivine and formation of products, which are identical whether formed during natural terrestrial exposure or continued reaction during curatorial storage under $N_2$ atmosphere after recovery in Antarctica [7,8]. Environmental control lapses during sample storage have been shown to exacerbate some of these effects [10].

**Evaporite minerals in and on carbonaceous chondrites:** Carbonaceous chondrites are especially vulnerable to elemental mobilization and formation of secondary minerals (usually evaporites) during exposure to water in ostensibly dry environments (Antarctic cold deserts; laboratory atmosphere) [9-12]. Weathering in the curatorial and / or laboratory environment has been documented for falls of several C chondrite groups. Reactive soluble species (sulfates) were remobilized, apparently by exposure to moisture in laboratory atmosphere, during curatorial storage of the CI carbonaceous chondrite Orguiel [12]. Carbonate minerals have been similarly redistributed during storage of Vigarano (CV3) [13]. A strong correlation exists between the Mineralogical Alteration Index (MAI) [14] for non-Antarctic CM chondrite falls and the year of the fall – the longer ago the fall, the higher the MAI [15]. MAI has recently been shown to vary with the Chemical Index of Alteration, a widely used chemical-weathering index [11]. This suggests that MAI varies with terrestrial weathering of CM chondrites, even falls weathered after recovery and during storage [11].

**Organic-clay interactions in carbonaceous chondrites:** The search for organic carbon compounds indigenous to the parent bodies of meteorites and intentionally returned samples from solar system objects is a high priority for planetary materials

research [16]. C2 carbonaceous chondrite falls with phyllosilicates in their matrices adsorbed volatile organic-carbon compounds from laboratory sample-storage materials within one day, between sample preparation and analysis [16]. Similar contamination affected identically processed serpentine, smectite, and silica gel, but no such contamination occurred in an identically processed phyllosilicate-free carbonaceous chondrite of a different compositional class [16]. Phyllosilicate minerals are expected to be among the possible host phases for organic-carbon compounds in samples to be returned by future sample return missions to carbonaceous-chondrite-like asteroids, but the same phyllosilicates will be attractive host phases for terrestrial contaminant organic-carbon compounds [11,16]. The same reactivity (due to high surface-area/volume ratio) that makes fine-grained minerals of carbonaceous chondrites the most promising recorders of parent-body alteration may also make the fines the parts most vulnerable to reactions with terrestrial moisture, oxidants, and contaminants, even in museum and laboratory settings.

**Summary:** Improved scientific understanding is expected to result from acquisition of samples directly from their parent bodies, without the intermediate mineral-modifying processes that affect meteorites during atmospheric entry, terrestrial weathering, and storage. However, intentionally returned samples containing water-soluble or redox-sensitive minerals and / or organic-chemical compounds will be just as vulnerable to post-acquisition modification of their indigenous inventory of such compounds by reactions with terrestrial moisture, oxidants, and contaminants as are meteorites. Redistribution of soluble minerals and their constituent elements and isotopes, and modification of organic-chemical compounds, complicate the interpretation of these minerals and molecules and their significance for pre-terrestrial phenomena.

Low-preservation potential aqueous alteration features (e.g., evaporite minerals in their indigenous hydration states) will not survive intentional excursions of T and relative humidity during thermal sterilization for planetary protection, or excursions in which environmental controls for the sample-return container and laboratory containment protocols are either limited by design or fail. Temperature, relative humidity, and redox conditions must be strictly controlled during sample return missions, continuing after sample return to include curation, storage, and examination, if preservation of soluble and hydrated minerals and / or organic molecules in their indigenous textures, distributions, associations, and hydration and oxidation states are goals of sample return. Experience with chondrite falls suggests that special care will be required during and after preliminary examination and after distribution of returned small-body samples to investigator laboratories in order to ensure that sample integrity carefully preserved during sample acquisition and return is maintained after sample allocation and distribution.

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**METASTABLE MINERALS AS A BIOSIGNATURE.** F. Westal1l, B. Cavalazzi1,2, C. Andreazza3, F. Foucher1, J-N. Rouzaud4, L. Lemelle5, A. Simionovici6, 1Centre de Biophysique moléculaire-CNRS-OSUC, Orléans, France (frances.westall@cnrs-orleans fr), 2Univ. Johannesburg, South Africa, 3Centre de Recherche de la Matière Divisée-CNRS, Orléans, France ; 4ENS-Géologie, Paris, France, 5ENS-Géologie Lyon, France, 6ISTE-Grenoble, France

**Introduction:** Certain minerals and/or mineral features are considered to be biosignatures. Minerals directly or indirectly precipitated through microbial activity, such as carbonate, Fe oxides, phosphates to name but a few, are well known. Other kinds of microbial influence on minerals include corrosion, element leaching and structural defects (stunted crystal faces, stunte crystal growth) induced by the fixation of organic molecules on the growing mineral crystal faces [1]. Recently, metastable minerals associated with other fossilised traces of life have been described from the rock record [2-4]. Metastable minerals could be a novel addition to the known range of biosignatures of relevance for the forth coming in situ missions to mars..

In this contribution we describe metastable aragonite associated with a 3.3 Ga-old microbial mat from the Barberton Greenstone Belt.

**Metastable aragonite in a 3.3-Ga-old microbial mat from Barberton, South Africa:** We identified aragonite in the calcified, degraded organic matrix of a 3.3 Ga-old photosynthetic microbial mat that has undergone regional lowermost greenschist metamorphism [3]. The aragonite occurs as 5-10 nm-sized crystallites that have nucleated onto the alveolar organic substrate (Fig. 1). It was identified by HRTEM analysis of the carbonate crystallites that documented interplanar distances of 0.335 nm, corresponding to the d111 interplane of the carbonate phase aragonite. Fast Fourier Transform imaging of the nanocrystals shows a cloudy-spotty ring (corresponding to d = 0.335 nm), typical of the diffraction pattern generated by multiple crystallites with variable rotational orientation. In composition, apart from Ca as the cation, it is characterised by minor Mg, as well as trace amounts of Fe and Cr. The addition of Mg and other trace elements is common in aragonite.

The aragonite most likely precipitated as a result of the activity of heterotrophic degradation of the organic matter in the mat by sulphur reducing bacteria. Normally aragonite converts rapidly to calcite but, in the case of the 3.3 Ga-old microbial mat, recrystallisation was inhibited by the organic matrix in which the carbonate phase precipitated. A similar situation was found with 2.7 Ga-old aragonites associated with microbial organics [2]. An additional factor in the preservation of the aragonite is that the microbial mat was preserved by permeation by hydrothermal silica as it was living which effectively "locked" the already-formed minerals and prevented further alteration.

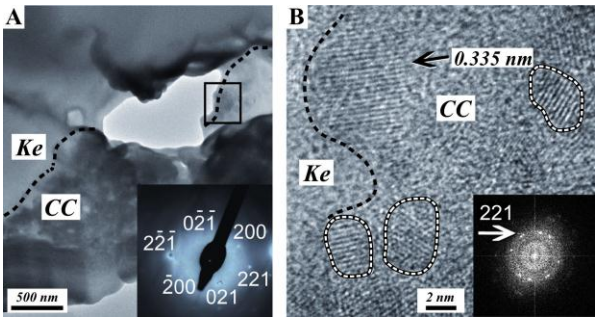


*Fig. 1. Metastable aragonite embedded in the organic matrix of a 3.3 Ga-old microbial mat from Barberton, South Africa*

**Discussion and conclusion:** Aragonite is typically an unstable phase that rapidly converts to calcite and is therefore rare in the geological record [2,5]. However, we have shown that aragonite nanocrystals can survive lowermost greenschist metamorphism and 3.3 Ga of geological history. The microbial mat formed in situ on the early Archaean sediment surface and was calcified and then immediately silicified in situ [3,4]. The syngenicity of mat formation, calcification and silicification is documented by structural and compositionnal mat characteristics, the age of the organic molecules (mostly aromatic structures), the nucleation of the crystallites onto the organic matrix, and the composition of the aragonite (the ambient environment was enriched in elements such as Fe and Cr leached from the ultramafic/mafic volcanics).

The association of a metastable mineral phase with organic carbon could be considered to be sufficient indication of potential biogenicity for a rock containing these signatures to be selected for more detailed analysis in situ on Mars and for return to the Earth. This scenario is of relevance for the forthcoming international 2018 mission to Mars in which arm mounted instruments may be used to preselect samples for more detailed mineralogical, elemental and organic analysis in the Pasteur laboratory.

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**ANCIENT BIOSIGNATURES IN ROCKS AND THEIR RELEVANCE IN THE SEARCH FOR EXTRATERRESTRIAL LIFE.** F. Westall[l], B. Cavalazzi[1,2], F. Foucher[1], A. Hubert[1,4], [1]Centre de Biophysique moléculaire-CNRS-OSUC, Orléans, France (frances.westall@cnrs-orleans.fr), [2]Univ. Johannesburg, South Africa, [3]ENS-Lyon, France, [4]ISTE-Grenoble, France, [5]ISTO-CNRS-Orléans, France.

**Introduction:** The search for biosignatures in rocks, including extraterrestrial materials, requires solid understanding of the nature of biosignatures, their preservation, and their identification. The most ancient traces of life on Earth, which formed at a time when life may still have existed at the surface of Mars (if it ever appeared, *i.e.* ~3.5 Ga [1,2]), is therefore of enormous benefit in the search for ancient traces of life, for example on Mars. The significance of these ancient life forms in terms of environment of formation (= local habitability), early stage of evolution, mode of biosignature preservation, as well as the methods used for biosignature identification, is extremely important in helping to plan strategies for *in situ* analysis on another planet, choice of sample for return to Earth, and analysis of returned samples in terrestrial laboratories.

**Ancient biosignatures:** The most immediately evident biosignatures in rocks ~3.5 Ga-old are macroscopic to microscopic vestiges of photosynthetic microbial mats (mats and stromatolites, [3]). Photosynthesis is, however, a very evolved metabolic strategy. Given the limited habitability conditions on early Mars, when life could possibly have flourished, it is unlikely that such an evolved form of energy transfer could have developed independently on Mars [2]. Nor is it likely that such organisms could have been transported from the Earth to Mars in meteorites [4]. On the other hand, the abundant volcanic and organic primary materials on Mars could have supported primitive metabolisms, such as chemotrophy. Note that the environmental conditions on the early Earth were very different to those of the present day planet [1,5]: basically anoxic, warmer ocean water temperatures, slightly acidic pH, silica-staurated seawater, etc.

Chemotrophic signatures in the ancient terrestrial rocks are subtle and relatively difficult to identify because of the nature of the microorganisms that produced them and their modes of preservation. The microorganisms were small (Fig. 1), heterogeneously distributed, and contributed little carbon biomass. They formed colonies on the surfaces of volcanic rocks and particles, leaving corrosion patterns in the vitreous surfaces of pillow basalts [6] and volcanic shards (Fig. 2 [7]). Preservation of the morphological, chemical and isotopic signatures was by silicification (some biostructures were calcified before silicification, [8]) which, while very effectively preserving microbial morphologies and chemical and isotopic signatures (silica, for example, being less "leaky" than carbonate), at the same time, significantly diluted these signatures [8,9]. The mode of preservation of the biosignatures therefore places additional constraints on their detectability.

For effective interpretation, any study of ancient biosignatures needs to be placed in different levels of environmental context [5]: from the regional to local environment of deposition, e.g. deep basin, littoral, hydrothermal; to the microbial scale environment, e.g. rock/mineral surface, sediment surface. The former must necessarily be evaluated on Mars but the microenvironment will be analysed in the returned sample since it is the immediate environment of the potential biosignature that is of importance.

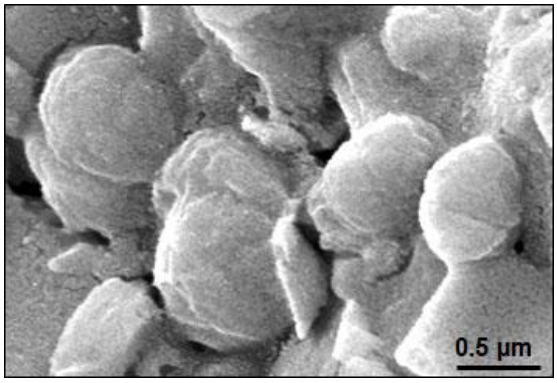


*Fig. 1. ~3.5 Ga-old silicified chemolithotrophs in volcanic sediments from the Pilbara [5].*

**Analytical implications:** Methods used today to study the structure and composition of the organic molecules, the isotopic signatures and the morphology of the fossil microbial traces are wide ranging and highly sophisticated. Given the small size of the microbial structures and their chemical traces, *in situ* observation/analysis on the micron to nanometer scale is desirable in order to better evaluate the biogenicity of the features. This induces constraints on instrument detection levels and resolution.

*Morphological biosignatures:* Fossilised chemolithotrophic microorganisms in ~3.5 Ga-old rocks from the Pilbara in Australia are ~0.5-1 µm in size (Fig. 1 [5]). They may leave corrosion features in the surfaces of rocks/minerals of the order of a few microns in size. Possible confusion of morphological signatures with

similar structures formed abiogenically means that nm-scale structural details as well as other lines of biogenic evidence are needed for support [5].

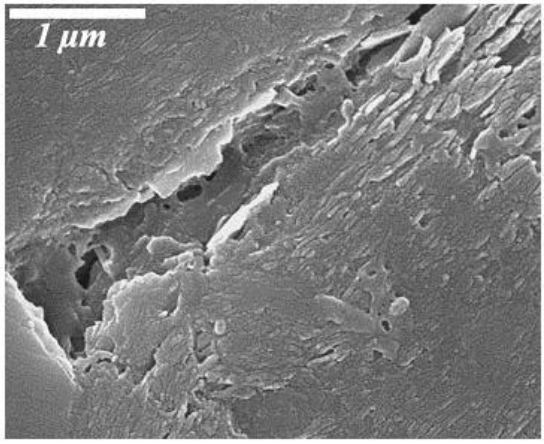


*Fig. 2. Corrosion tunnel in a 3.5 Ga-old volcanic shard from the Pilbara [5].*

The fossil chemolithotrophs are large compared to hypothesised primitive cells, perhaps similar to viruses in size. Martian life may have been very small and may not have reached the size of present day (or even Early Archaean) chemotrophic life forms. Viruses contribute hugely to the present microbial but, biomass until recently, have generally been analytically "invisible". Although they can be fossilised (Fig. 3 [10]), the resulting structures (of the order of 50-100 nm) would be very difficult to identify.

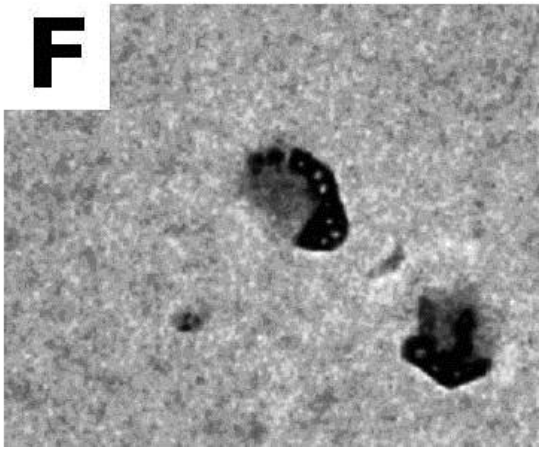


*Fig. 3. Silicified viruses [10].*

*Organic molecules:* The TOC in early Archaean sediments is generally low (0.01-0.05%) but can be up to 0.1-0.5% in particularly rich sediments. Apart from dilution due to fossilisation and the fact that they have undergone burial metamorphism (prehnite-pumpellyite to lowermost greenschist facies), these very old molecules are significantly degraded. Most are aromatic fragments of the original macromolecules |8,11]. Although they have many similarities with abiogenic PAHs, they also exhibit more complexity than the latter.

Microbial organic matter typically contains either structurally important metals and/or "opportunistically"-chelated metals. Excess concentrations of certain metals compared to the background levels may also contribute to interpretations of biogenicity.

*Metabolic signatures:* Isotopic fractionation of certain bio-essential elements, such as C, N, Fe etc., is the most commonly used signature of past microbial metabolism but other signatures include biominerals and corrosion or leaching effects in adjacent rocks and minerals.

**Conclusions:** The search for biosignatures in returned martian materials will really be like searching for a needle in a haystack. In the first place, there is a strong likelihood that the life forms may be even more primitive and smaller than the oldest forms of life on Earth. This means that microbial TOC in the sediments/rocks will be limited and also that the biosignatures are likely to be heterogeneously distributed. The technological challenges for their identification are therefore strong but continued advances in the level of instrumental resolution are promising. The conclusion is that it will probably only be in samples returned from Mars that we will be able to definitively identify *bona fide* biosignatures (if they exist!).

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**MELOS LIFE SEARCH PROPOSAL: SEARCH FOR MICROBES ON THE MARS SURFACE WITH SPECIAL INTEREST IN METHANE-OXDIZING BACTERIA.** A. Yamagishi[1], Y. Yoshimura[2], H. Honda[3], A. Miyakawa[4], Y. Utsumi[5], T. Itoh[6], T. Naganuma[7], S. Ohno[8], R. Ishimaru[8], S. Sasaki[9], T. Kubota[10], T. Satoh[10], K. Tonokura[11], H. Miyamoto[11], [1]Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo, Japan, [2]Tamagawa University, Tokyo, Japan, [3]Nagaoka University of Technology, Niigata, Japan, [4]Shizuoka University, Shizuoka, Japan, [5]University of Hyogo, Hyogo, Japan, [6]RIKEN BioResource Center, Saitama, Japan, [7]Hiroshima University, Hiroshima, Japan, [8]Chiba Institute of Techonology, Chiba, Japan, [9]NAOJ, Japan, [10]ISAS/JAXA, Kanagawa, Japan, [11]The University of Tokyo, Tokyo, Japan,

**Introduction:** Among the planets and giant satellites in our solar system, the characteristics of Mars are most similar to those of Earth. This may suggest that it may be possible for life similar to terrestrial life to arise and to survive on Mars.

In the Viking missions conducted by NASA in 1976 the Gas Chromatography / Mass Spectrometer (GCMS) Experiment showed the absence of detectable organic compounds in the upper 10 cm of surface soil[1]. The results of the experiments were interpreted to indicate the presence of oxidants that decomposed the organic compounds, and no organisms were present within the detection limits of the experiments [2].

However, the results of the Viking experiments have been reexamined. Biomolecules such as amino acids could not be detected if living cells were present at levels less than $10^7$ cells per gram[3]. Thus some organic compounds may be present on Mars, although compounds near the surface may be destroyed by ionizing radiation[4,5].

The Planetary Fourier Spectrometer onboard the Mars Express spacecraft detected methane at a concentration of approximately 10 ppbv in global abundance[6,7]. Similar abundance was observed by the Fourier Transform Spectrometer at the Canada-France-Hawaii Telescope[8]. Methane was released in large plumes and peak amounts were observed during Martian summer with high-dispersion infrared spectrometers[9].

Here we propose a new life detection project on Mars to search for methane-oxidizing microbes by fluorescence microscopy combined with amino acid analysis and mass spectrometry[10]. We propose to search for "cells" from a depth of about 5 - 10 cm below the surface, which is feasible with current technology. Microscopic observation can be done using low mass equipment with low electric power consumption, and has the potential to detect single "cells". The subsequent analysis of amino acids will provide the information needed to define the origin of the "cell".

**Survivability of Life in the Mars Environment:** Physical and chemical limits for terrestrial life have been major foci in astrobiology[11,12], and are summarized in ref. [10].

UV radiation on the Mars surface can be estimated to be 20 W $m^{-2}$. If the same shield performance holds for other ionizing radiations[10], ca. 1.2 mGy $day^{-1}$ as determined during the 2001 Mars Odyssey mission, a dose of 0.4 mGy $day^{-1}$ is estimated for the Mars surface. This dose of ionizing radiation is far below the effective dose to kill radio-resistant microbes.

Results of the Viking biological experiments were interpreted to indicate the presence of highly oxidizing compound, which could be generated by photochemical reactions and/or dust storms[13]. Many microorganisms show high tolerance to oxidative conditions, and thus any possible Martian life may not be damaged too seriously[14]. Extended survival of several organisms and aminoacids under simulated martian surface conditions has been reported[15].

**What is Needed for Life:** "Liquid water" is the simplest and most universal answer. However, many microorganisms can survive in a vacuum for many years. Not only spore forms but also vegetative cells can be stored alive in a vacuum under dessicated conditions.

Another requirement to sustain life is Gibbs free energy[10]. In general, we often refer to it simply as "energy". Animals depend on food to sustain life. Oxygen is also needed to sustain animal life. These are the two substrates needed to obtain Gibbs free energy for animals. Free energy can be also obtained via other metabolic pathways, such as photosynthesis in plants, and chemosynthesis in some microorganisms. Chemosynthetic microorganisms can obtain free energy from the reaction between reducing compounds and oxidative compouns.

Combining these factors, anywhere in the Martian environment where we can find these three components, water molecules, reducing compounds such as $H_2$, $H_2S$ and $CH_4$, and oxidative compounds such as ferric oxide, sulfate and perchlorate, could be an environment where life can be sustained for long periods of time, if other factors such as temperature, pressure, UV and other radiations permit.

**Methane Oxidizing Bacteria on Earth:** Methane can be used as a primary energy source by a number of

Bacteria and Archaea. Most known methane-oxidizing bacteria are aerobic; however, some evidence, mostly indirect, points to the existence of anaerobic methanotrophs[16]. Recently, a microbial consortium that is capable of using manganese (birnessite) and iron (ferrihydrite) to oxidize methane has been predicted in marine methane-seep sediments in the Eel River Basin in California[17]. Thus, there are several mechanisms of methane oxidation carried out by Bacteria and Archaea on Earth, and possibly on Mars.

**How to Find Microbes on Mars:** Fluorescent microscopy is a method to detect localized biosignatures *in situ*. Biosignatures are labeled with fluorescent dyes. Stained objects are observed with an epifluorescence microscope with a resolution of 1 $\mu$m. This method is highly sensitive. Many fluorescent dyes are commercially available and used routinely to study terrestrial microorganisms[18,19]. Analytical procedures are simple and can be done in a short time. Dye solutions will be sprayed onto samples and digitized images will be obtained using a CCD camera.

The combination of pigments used will be optimized to detect biological characteristics that define life. The "cell" should be surrounded by an impermeable membrane to define "self" and "non-self" and to distinguish inside from outside. The presence of this defining characteristic will be tested detecting the boundaries using a combination of membrane permeable and impermeable pigments.

The second characteristic is metabolism. All life forms depend on free energy, obtained from metabolism. Metabolism, in turn, consists of a complex series of biological reactions called metabolic pathways, which are catalyzed by enzymes. We plan to detect esterase, one of the most commonly found enzymes in cells on Earth.

The third characteristic is division or proliferation of a "cell". Because it is not easy to find appropriate conditions for proliferation, direct observation of the proliferation process is less feasible. Instead, we will target the genetic molecules needed for reproduction of the genetic information.

Upon identification of candidate "cells" by fluorescence microscopy, they will be analyzed by second stage analytical process, possibly in another misson. In the second stage, the "cells" will be hydrolysed.

Living cells on Earth consist of 70% water and 15% protein. Cells contain many types of proteins, from as many as several thousand proteins in prokaryotes, to several tens of thousands in eukaryotes, each having a molecular weight from a few thousand to several hundred thousand. The molecular weight spectra are too complex to be resolved by any type of mass spectral detectors. However, once proteins are hydrolyzed, they produce a mixture of 19 chiral-specific amino acids and glycine, which has no optical isomer. A specific set of 20 amino acids is commonly found in all cells on Earth. Based on research on chemical evolution, which must have occurred before the origin of life, amino acids are known to be abiotically produced in a wide range of possible prebiological environments. Accordingly, there is a fair chance that Martian "cells" contain polymers of amino acids. However, the number, types and chirality of the amino acids may not be the same as those in living cells on Earth. The number and characteristics of amino acids will be analyzed in the second stage of "cell" analysis.

**Conclusion:** We propose to search for microbes on Mars, 5 to 10 cm below the surface. The first effort should be to identify locations where methane is emitted from underground. The rover will approach the methane-emitting site, where soil will be collected and analyzed. A combination of fluorescent dyes will be used to detect candidate "cells" using a fluorescence microscope[10]. Possibly in another mission, putative "cells" will be hydrolyzed and analyzed by HPLC and/or mass spectral analysis to define the characteristics of the candidate "cells", which will indicate the origin of the candidate "cells" [10].

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**SEARCHING FOR ORGANICS PRESERVED IN 4.5 BILLION YEAR OLD SALT**

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**Introduction**: Our understanding of early solar system fluids took a dramatic turn a decade ago with the discovery of fluid inclusion-bearing halite (NaCl) crystals in the matrix of two freshly-fallen brecciated H chondrite falls, Monahans (1998, hereafter simply "Monahans") (H5) and Zag (H3-6) [1, 2]. Both meteorites are regolith breccias, and contain xenolithic halite (and minor admixed sylvite – KCl, crystals in their regolith lithologies. The halites are purple to dark blue, due to the presence of color centers (electrons in anion vacancies) which slowly accumulated as $^{40}$K (in sylvite) decayed over billions of years. The halites were dated by K-Ar, Rb-Sr and I-Xe systematics to be 4.5 billion years old [2-4]. The "blue" halites were a fantastic discovery for the following reasons: (1) Halite+sylvite can be dated (K is in sylvite and will substitute for Na in halite, Rb substitutes in halite for Na, and I substitutes for Cl). (2) The blue color is lost if the halite dissolves on Earth and reprecipitates (because the newly-formed halite has no color centers), so the color serves as a "freshness" or pristinity indicator. (3) Halite frequently contains aqueous fluid inclusions. (4) Halite contains no structural oxygen, carbon or hydrogen, making them ideal materials to measure these isotopic systems in any fluid inclusions. (5) It is possible to directly measure fluid inclusion formation temperatures, and thus directly measure the temperature of the mineralizing aqueous fluid [1].

In addition to these two ordinary chondrites halite grains have been reliably reported in several ureilites [5], an additional ordinary chondrite (Jilin) [6], and in the carbonaceous chondrite (Murchison) [7], although these reports were unfortunately not taken seriously. We have lately found additional fluid inclusions in carbonates in several additional carbonaceous chondrites. Meteoritic aqueous fluid inclusions are apparently relatively widespread in meteorites, though very small and thus difficult to analyze.

**Isotopic Work**: Last year we reported results of the first O and H isotopic measurements of the aqueous fluids present within the Monahans and Zag halites [8]. Variations of δD of the fluids range over −330(90; 2σ) to +1200(90)‰ for Monahans and −300(96)‰ to +90(98)‰ for Zag. Δ17O of the fluids range over −16(22)‰ to +18(10)‰ for Monahans and +3(10)‰ to +27(11)‰ for Zag. The variations are larger than the reproducibility of standard analyses and suggest that isotope equilibria were under way in the fluids before trapping into halite. The mean values of δD and Δ17O are +290‰ and +9‰, respectively. The mean values and the variations of the fluids are different from the representative values of ordinary chondrites, supporting our view that the aqueous fluids were not indigenous to the H chondrite parent-asteroid(s) but rather were exogenous fluids delivered from somewhere else. The implication is that the halites travelled from another early solar system body (or bodies) that was (were) undergoing cryovolcanism. It is interesting that the aqueous fluid δD is intermediate in composition between primitive meteorites and Oort cloud comet coma water [8].

**Organics**: Simple organic structures such as nanoglobules are commonly present in astromaterials, even those that have been metamorphosed to a significant degree [9]. For example, they are present in CI chondrites which we know have been subjected to boiling aqueous fluids. Our previous work on the aqueous fluid inclusions in meteoritic halite has demonstrated that the fluids were never at temperatures greater than ~25C – far lower than CI

chondrites, and more in line with most CM chondrites [1]. Terrestrial halite commonly contains trapped organic structures, which can be perfectly preserved for hundreds of millions of years at least (the record is 250 million years so far [10]). We thus investigated whether complex organic compounds and structures were trapped within the growing meteoritic halites alongside fluid inclusions (and in fact may have served as nucleation points for the fluid inclusions.

We have already found that the meteoritic halites have inclusions that fluoresce under short- and long-wave UV radiation which is the most sensitive text for the presence of potential organics (G. Cody, personal communication, 2009), although the fluorescing phases don't have to be organics. We therefore examined freshly-cleaved surfaces of meteoritic halite crystals for trapped organic compounds by confocal Raman Microscopy in Andrew Steele's Lab at the Carnegie Institution [11]. Examination of Monahans and Zag halite grains with Raman imaging reveals the presence of small (<10 μm), primarily metal grains with oxidation/hydroxilation products and minor phases inconsistent with H-chondrite mineralogy that must arise from the halite grains' originating body. The grains are generally subrounded and composed largely of metal, magnetite and another phase which is probably fine-grained lepidocrocite (FeO(OH)). Identification of lepidocrocite carries an element of uncertainty at the time of this writing due to broad spectral features shared with clays and glassy phases, but in advance of data from other techniques the identification as lepidocrocite is reasonable given the inclusions' apparent history as largely metallic clasts altered by interaction with brine. Additional phases include forsteritic olivine (Fo≈98), pyroxenes, a feldspar grain with Raman spectral affinity to anorthoclase We indeed identified organics in the halite as well, including macromolecular carbon (MMC). One inclusion features aliphatic material with Raman spectral features consistent with a mixture of short chain aliphatic compounds. Our search for organics is continuing to other halite grains, and the fluid inclusions within meteoritic carbonates.

**Parent Bodies**: The minerals and compounds discovered thus far within Monahans/Zag halites are indicative of an originating body at least partly composed of equilibrated igneous materials (high Fo olivine, possibly the metal) which was subjected to aqueous alteration (the halite parent brine) and containing a light organic component (the short-chain aliphatic compounds). Ultimately, this material was ejected from the originating body with little or no disruption, as evidenced with the presence of surviving fluid inclusions. An actively geysering body similar to modern Enceladus [12] is a reasonable analogue. Also, the originating body may have been in close proximity to the H chondrite parent in order to generate the number of halite grains seen in Monahans and Zag. Other candidates for Monahans/Zag halite parent bodie(s) include a young Ceres with its possible liquid ocean or other similar C-, P- or D-class asteroids [13], and Main Belt comet(s) [14].

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# NOTES